CONTINUATION APPLICATION

FOR

UNITED STATES LETTERS PATENT

TITLE: BIOACTIVATED DIAGNOSTIC IMAGING CONTRAST AGENTS

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BIOACTIVATED DIAGNOSTIC IMAGING CONTRAST AGENTS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a Continuation of United States Patent Application No. 08/823,643, filed March 25, 1997, which claims the benefit of United States Provisional Application No. 60/014,448, filed April 1, 1996.

TECHNICAL FIELD OF THE INVENTION

This invention relates to improved diagnostic agents for Magnetic Resonance Imaging (MRI) and optical imaging. These agents permit the sensitive detection of a specific bioactivity within a tissue. This invention also relates to pharmaceutical compositions comprising these agents and to methods of using the agents and compositions comprising the agents.

BACKGROUND OF THE INVENTION

Diagnostic imaging techniques, such as MRI, x-ray imaging, nuclear radiopharmaceutical imaging, ultraviolet/visible/ infrared light imaging, and ultrasound imaging, have been used in medical diagnosis for a number of years.

Commonly used contrast materials include organic molecules, metal ions, salts or chelates, particles (particularly iron particles), or labeled peptides, proteins, polymers or liposomes. After administration, these agents may non-specifically

diffuse throughout body compartments prior to being metabolized and/or excreted; these agents are generally known as non-specific agents. Alternatively, these agents may have affinity for a particular body

5 compartment, cell, organ, or tissue component; these agents can be referred to as targeted contrast agents.

Contrast agent-enhanced diagnostic imaging procedures desirably increase the contrast between normal and pathological tissue in such a way as to

10 provide two basic classes of information:

1) Detection Data. This includes data necessary to determine whether an abnormality is present in the imaged tissue and the degree to which it is present. The ability to provide this class of

15 information relates to the "sensitivity" of the imaging procedure.

2) Differential Diagnosis Data. This includes data necessary to identify with precision the type of abnormality present. The ability to provide

20 this class of information relates to the "specificity" of the imaging procedure. Specificity is necessary for making an accurate prognosis of the patient's condition and a plan of therapy. For example, although current procedures may be able to detect a tumor, generally

25 they are inadequate to determine whether the tumor is benign or malignant, whether the tumor is likely to metastasize, or whether the tumor is responding to therapy. Such determinations require some knowledge of the specific biochemical state of the tissue.

30 A number of approaches have been presented to create targeted contrast agents. U.S. patent no. 4,880,008, incorporated herein by reference, describes MRI contrast agents which exhibit higher signal, or relaxivity, when they bind non-covalently to serum

proteins, such as human serum albumin. For this class
of agents, the relaxivity is related to the percent of
the contrast agent bound to protein and is typically
five to ten times higher than that observed for agents
5   that do not bind proteins. In co-pending U.S.
application ser. no. 08/382,317 (filed February 1,
1995), incorporated herein by reference, blood half
life extending moieties ("BHEMs") are added to the
protein-binding contrast agents. The resulting agents
10   exhibit enhanced or altered signal for a longer period
of time in blood relative to agents lacking the BHEM,
rendering these materials especially useful for
vascular imaging.

U.S. patent no. 4,899,755, incorporated
15   herein by reference, describes MRI contrast agents
which are preferentially taken up in normal
hepatocytes, resulting in contrast enhancement between
normal and abnormal liver tissue.

Another targeting approach is based on
20   conjugation of contrast agents to proteins, antibodies
or other biomolecules which are known to interact with
cell surface receptors, intracellular receptors,
transporters, or other biochemical constituents. See,
e.g., U.S. Patent No. 5,171,563. However, such
25   targeting usually involves a one-to-one interaction
between the conjugated agent and the biochemical
target, which is often present in relatively low
concentrations (frequently nanomolar). Consequently,
the number of targeted contrast agent molecules which
30   accumulate in a particular tissue using this approach
is limited. For imaging modalities where a significant
concentration of agent molecules is often needed for
detection (e.g., > 1 uM), such as MRI and optical

imaging, this "one-to-one" approach is generally too insensitive to be useful.

Attempts to image the biochemical state of tissues include radiopharmaceutical applications, where
5   certain imaging agents are retained in a particular tissue. For example, the positron-emitting ^{18}F-labeled fluorodeoxyglucose is transported into the brain by passive diffusion, where it is phosphorylated and retained within brain tissue, resulting in an
10 indication of glucose metabolism (see M. Blau, Seminars in Nuclear Medicine, Vol. XV, No. 4 (October), 1985). Similarly, a technetium-99m labeled nitroimidazole is reported to be preferentially retained in ischemic heart (see Y.-W. Chan et al., Proceedings of the 41st
15 Annual Meeting of the Society of Nuclear Medicine, June 5-June 8, 1994, J. Nuclear Medicine (1994), Volume 35, Abstract No. 65, p. 18P). However, in these cases, the signal from the radiopharmacetical remains constant (i.e., each radioisotope has a characteristic,
20 invariant decay and energy of the particles emitted) and is not affected by either biomodification or preferential retention in a tissue. The specificity and sensitivity of the information which can be obtained by this technique is limited.
25   There remains a need for contrast agents with improved specificity and sensitivity. In particular, there remains a need for targeted MRI and optical contrast imaging agents that exhibit enough signal enhancement or signal alteration in response to the
30 presence of specific bioactivities to be useful in diagnosing the presence of those bioactivities.

SUMMARY OF THE INVENTION

The present invention provides novel improved diagnostic MRI and optical imaging agents for the sensitive detection of a specific bioactivity within a tissue, and pharmaceutically acceptable derivatives thereof. The imaging agents are prodrug contrast agents which are bioactivated in vivo in the presence of the specific bioactivity. Where the bioactivity is catalytic (e.g., stemming from enzyme activity), a large number of activated contrast agent molecules is generated for every unit of bioactive substance. The bioactivated form of the imaging agent exhibits increased binding affinity for one or more proteins. compared to the prodrug, and this change in binding affinity causes a detectable change in the signal characteristics in the imaging agent. This detectable signal change increases the signal (or image) contrast between tissues which contain the targeted bioactivity and those which do not, which thus reflects the presence of the targeted bioactivity.

It is an object of this invention to provide novel compounds that are useful as contrast agents in MRI and optical imaging. It is also an object of this invention to provide pharmaceutical compositions comprising these compounds. It is a further object of this invention to provide methods for using these compounds and compositions comprising them in MRI and optical imaging.

DETAILED DESCRIPTION OF THE INVENTION

In order that the invention herein described may be more fully understood, the following detailed description is set forth.

The novel prodrugs of the present invention
are designed with three constraints in mind: 1) they
must have one or more specific sites in their structure-
that can become modified in vivo by a specific
5   bioactivity; 2) the modified form of the imaging agent
generated by this bioactivity must bind to one or more
proteins to a greater degree than the prodrug; and
3) the signal characteristics of the imaging agent must
be altered when it binds to a protein.

10   For the present invention, image contrast
between normal and abnormal tissue generally requires
the bioactivity in one of the tissues to be higher than
that in the other. If abnormal tissue expresses a
greater concentration of bioactivity than normal
15   tissue, then abnormal tissue will convert more prodrug
contrast agent to the activated form than will normal
tissue (provided that similar concentrations of prodrug
are present in both tissues). In the specific example
where increased protein binding by the activated
20   contrast agent generates a more intense signal, the
presence of bioactivity results in the image (or
signal) being detected as a "hot spot." Conversely, if
the abnormal tissue expresses the lesser bioactivity,
then abnormal tissue will have a relatively lower
25   concentration of bioactivated contrast agent. In this
case, if the increased protein binding by the activated
contrast agent generates a more intense signal, the
presence of bioactivity results in image (or signal)
being detected as a "cold spot."

I.   Definitions

Listed below are definitions of terms used to describe the present invention. These definitions apply to the terms as they are used throughout the specification unless otherwise indicated.

The term "aliphatic," as used herein alone or as part of another group, denotes optionally substituted, linear and/or branched chain, saturated or unsaturated hydrocarbons, including alkenyl, alkynyl, cycloalkyl and cycloalkenyl hydrocarbons.

The term "alkyl," as used herein alone or as part of another group, denotes optionally substituted, linear and/or branched chain saturated hydrocarbons.

The terms "alkoxy" or "alkylthio" denote an alkyl group as described above bonded through an oxygen linkage (-O-) or a sulfur linkage (-S-), respectively. The term "alkylcarbonyl," as used herein, denotes an alkyl group bonded through a carbonyl group. The term "alkylcarbonyloxy," as used herein, denotes an alkyl group bonded through a carbonyl group which is, in turn, bonded through an oxygen linkage.

The term "alkenyl," as used herein alone or as part of another group, denotes optionally substituted, straight and branched chain hydrocarbon groups containing at least one carbon to carbon double bond in the chain.

The term "alkynyl," as used herein alone or as part of another group, denotes optionally substituted, straight and branched chain hydrocarbon groups containing at least one carbon to carbon triple bond in the chain.

The term "cycloalkyl," as used herein alone or as part of another group, denotes optionally substituted, saturated cyclic hydrocarbon ring systems.

The term "cycloalkenyl," as used herein alone or as part of another group, denotes such optionally substituted groups as described above for cycloalkyl, further containing at least one carbon to carbon double

5   bond forming a partially unsaturated ring.

The term "aryl," as used herein alone or as part of another group, denotes optionally substituted, homocyclic aromatic groups.

The term "heterocyclic," as used herein alone

10 or as part of another group, denotes optionally substituted fully saturated or unsaturated, aromatic or non-aromatic cyclic groups having at least one heteroatom.

The term "acyl," as used herein alone or as

15 part of another group, denotes the moiety formed by removal of the hydroxyl group from the -COOH group of an organic carboxylic acid.

The term "bioactivity" includes changes in pH, redox potential, concentration of reactive species

20 such as free radicals, or the presence or level of enzymes or biomolecules (including RNA enzymes) that can promote modification or cleavage of one or more bonds in the prodrug. A "bioactivity" can comprise two or more types of biomolecules that together or

25 sequentially cause modification of the prodrug. More than one biomodification can occur to the prodrug (e.g., an enzymatic cleavage followed by simple hydrolysis or decarboxylation).

II. Structure of the Prodrug

30   The prodrugs of this invention must comprise three domains: an image-enhancing (or signal-generating) moiety ("IEM"), a modification site ("MS"), and a protein binding moiety (PBM).
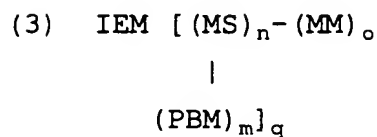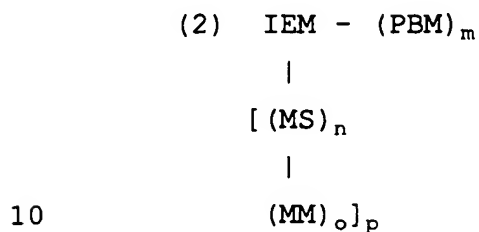
It is contemplated that the prodrugs of this invention may also comprise a physiologically compatible linker moiety (L) linking the functional domains. In general, L does not contribute
5 significantly to the protein binding or image enhancing functionality of the contrast agent. In some cases, the presence of L may be preferred based on synthetic considerations. In other cases, L may facilitate operation of the bioactivity at the MS. Examples of
10 L's include linear, branched, or cyclic alkyl, aryl, ether, polyhydroxyl, polyether, polyamine, heterocyclic, peptide, peptoid, or other physiologically compatible covalent linkages.

A preferred method of bioactivating the
15 contrast agents of this invention involves enzymatic cleaving of the prodrug at the MS (e.g., by an esterase, proteinase, phosphatase, etc.). In this case, the prodrugs of this invention further comprise a masking moiety (MM). The MM "masks" (or decreases) the
20 binding of the prodrug to the protein within the tissue desired to be imaged; once the MM is removed by cleavage at the MS, then the increased binding affinity of the agent is expressed. In this case, the target or substrate for the bioactivity (e.g., an amide bond) is
25 defined as the MS of the prodrug. This particular method of bioactivation results in the physical separation of at least two molecular fragments, one containing the IEM and PBM, and the other the MM.

The domains of the compounds of this
30 invention can be arranged in a variety of positions with respect to each other. While these domains can exist without any specific boundaries between them (e.g., the MS can be part of the IEM), it is convenient

to conceptualize them as separate units of the molecule.

For example, the following structures are contemplated:

5   (1)   IEM $-$ [$(PBM)_m$ $-$ [$(MS)_n$ $-$ $(MM)_o$ $]_p$ $]_q$

       (2)   IEM $-$ $(PBM)_m$
              |
          [$(MS)_n$
             |
10          $(MM)_o]_p$

       (3)   IEM [$(MS)_n$ $-$ $(MM)_o$
                |
             $(PBM)_m]_q$

wherein each of m, n, o, p and q are the same or
15 different, q, n, m and p can be greater than or equal to one, but not zero; and o can be greater than or equal to zero. Generally q, m, n and p are less than five. Most commonly, m, n, p and q are one and o is zero or one.

20   As used herein, the compounds of this invention are defined to include pharmaceutically acceptable derivatives thereof. A "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, salt of an ester, or other
25 derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an inhibitorily active metabolite or residue thereof. Particularly favored derivatives are those
30 that increase the bioavailability of the compounds of

this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a
5 biological compartment (e.g., the brain or lymphatic system) relative to the parent species).

Pharmaceutically acceptable salts of the compounds of this invention include those derived from pharmaceutically acceptable inorganic and organic acids
10 and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic,
15 ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Other acids, such as oxalic, while not in themselves pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in
20 obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g., sodium), alkaline earth metal (e.g., magnesium), ammonium and $N-(C_{1-4}$ alkyl$)_4^+$ salts.
25 The compounds of this invention contain one or more asymmetric carbon atoms and thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereomers. All such isomeric forms of these compounds are
30 expressly included in the present invention. Each stereogenic carbon may be of the R or S configuration. Although the specific compounds exemplified in this application may be depicted in a particular stereochemical configuration, compounds having either

the opposite stereochemistry at any given chiral center or mixtures thereof are also envisioned.

Combinations of substituents and variables envisioned by this invention are only those that result in the formation of stable compounds. The term "stable", as used herein, refers to compounds which possess stability sufficient to allow manufacture and which maintains the integrity of the compound for a sufficient period of time to be useful for the purposes detailed herein (e.g., therapeutic or prophylactic administration to a mammal or for use in affinity chromatography applications). Typically, such compounds are stable at a temperature of 40°C or less, in the absence of moisture or other chemically reactive conditions, for at least a week.

The compounds of the present invention may be used in the form of salts derived from inorganic or organic acids. Included among such acid salts, for example, are the following: acetate, adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptanoate, glycerophosphate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, oxalate, pamoate, pectinate, persulfate, 3-phenylpropionate, picrate, pivalate, propionate, succinate, tartrate, thiocyanate, tosylate and undecanoate.

This invention also envisions the quaternization of any basic nitrogen-containing groups of the compounds disclosed herein. The basic nitrogen

can be quaternized with any agents known to those of
ordinary skill in the art including, for example, lower
alkyl halides, such as methyl, ethyl, propyl and butyl -
chloride, bromides and iodides; dialkyl sulfates
5   including dimethyl, diethyl, dibutyl and diamyl
sulfates; long chain halides such as decyl, lauryl,
myristyl and stearyl chlorides, bromides and iodides;
and aralkyl halides including benzyl and phenethyl
bromides. Water or oil-soluble or dispersible products
10 may be obtained by such quaternization.

It should be understood that the compounds of
this invention may be modified by appending appropriate
chemical groups to enhance selective biological
properties. Such modifications are known in the art
15 and include those which increase biological penetration
into a given biological compartment (e.g., blood,
lymphatic system, central nervous system), increase
oral availability, increase solubility to allow
administration by injection, alter metabolism and alter
20 rate of excretion.

It should also be understood that the
compounds of this invention may adopt a variety of
conformational and ionic forms in solution, in
pharmaceutical compositions and in vivo. Although the
25 depictions herein of specific compounds of this
invention are of particular conformations and ionic
forms, other conformations and ionic forms of those
compounds are envisioned and embraced by those
depictions.

30   Further detailed description of the IEM, PBM,
MM, MS moieties is presented below. It should be
understood that compounds of this invention are
obtained by selecting among the various structures of

the moieties taught herein and incorporating them into the final compounds.

A.   Image Enhancing Moiety (IEM)

The IEM can be any chemical or substance that
5   provides the signal or contrast in imaging. When the
contrast agents of this invention bind to a protein,
there is a change in the IEM signal characteristic that
is detectable by the external detector. For optical
imaging, this can be a change in absorbance,
10 reflectance, fluorescence, an increase or decrease in
the number of absorbance peaks or any change in their
wavelength maxima, or any other change which by
external detection would correspond to a bound IEM.
Similarly, for MRI this can be a change in the induced
15 relaxation rates of water protons ($1/T_1$ or $1/T_2$) or any
other nearby nuclei, or a shift of one or more peaks or
alteration in signal intensity in the NMR spectrum of
either the IEM or peaks that appear from nuclei in the
binding site for the PBM.
20   For the purposes of this application, "MRI"
is understood to include magnetic resonance
spectroscopy techniques. The signals generated by
magnetic resonance spectroscopy generally provide
information in the form of a chemical shift (δ, in
25 units of ppm) instead of three dimensional images. The
chemical shift of a particular nucleus is related to
its chemical environment. When a prodrug is
bioactivated, the chemical shift of nuclei within the
prodrug will be altered.
30   The IEM can comprise an organic molecule,
metal ion, salt or chelate, cluster, particle
(particularly iron particle), or labeled peptide,
protein, polymer or liposome. For

ultraviolet/visible/infrared/fluorescence light
(optical) imaging, the IEM can also be any organic or
inorganic dye. Particularly useful inorganic dyes
include luminescent metal complexes, such as those of
5   Eu(III), Tb(III) and other lanthanide ions (atomic
numbers 57-71). See W. Dew. Horrocks & M. Albin,
Progr. Inorg. Chem. (1984), 31, pp. 1-104.
A particularly useful IEM is a
pharmaceutically acceptable metal chelate compound
10 consisting of one or more cyclic or acyclic organic
chelating agents complexed to one or more metal ions.
Metal ions preferred for optical imaging include those
with atomic numbers 13, 21-34, 39-42, 44-50, or 57-83.
Paramagnetic metal ions preferred for MRI include those
15 with atomic numbers 21-29, 42, 44, or 57-83.
If the IEM is a metal chelate, the metal
chelate should not dissociate to any significant degree
during the imaging agent's passage through the body,
including a tissue where it may undergo
20 biomodification. Significant release of free metal
ions can result in large MRI or optical signal
alterations, but may also be accompanied by toxicity,
which would only be acceptable in pathological tissues.
It is preferred that bioactivation not significantly
25 compromise the stability of the chelate so that the
metal complex can remain intact and be excreted. For
complexes in which kinetic lability is low, a high
thermodynamic stability (a formation constant
preferably of at least 10^{15} M^{-1} and more preferably at
30 least 10^{20} M^{-1}) is desirable to minimize dissociation
and its attendant toxicity. For complexes in which
kinetic lability is comparatively higher, dissociation
can be minimized with a lower formation constant, i.e.,
preferably 10^{10} M^{-1} or higher.

Formation constants of known coordination
complexes are generally less than 10^{60} and more
typically in the range of 10^{15} to 10^{40}. Coordination
complexes with suitable formation constants include

5   iron enterobactin (formation constant 10^{52}; W.R. Harris
et al., J. Am. Chem. Soc. (1981), 103, p. 2667), iron
MECAMS (formation constant 10^{41}; W.R. Harris et al. ,
J. Am. Chem. Soc. (1979), 101, p. 2213), gadolinium
diethylenetriamine pentaacetic acid ("DTPA") (formation

10   constant $10^{22.46}$; D.L. Wright et al. , Anal. Chem.
(1965), 37, pp. 884-892), gadolinium (1,4,7,10-
tetraazacyclotetradecene 1,4,7,10-tetracetic acid
("DOTA") (formation constant $10^{25.3}$; K. Kumar et al.,
Inorganic Chemistry (1993), 32, pp. 587-593),

15   gadolinium DTPA-BMA (formation constant $10^{16.9}$; W.P.
Cacheris et al., Mag. Res. Imag. (1990), 8 pp. 467-481)
and gadolinium EDTA (formation constant $10^{17.3}$; D.L.
Wright et al., Anal. Chem. (1965) 37, pp. 884-892).
Formulations of gadolinium DTPA, DOTA, and DTPA-BMA are

20   used clinically as MRI contrast agents.

Toxicity is also a function of the number of
open coordination sites in the complex. The fewer
coordination sites, the less tendency there is,
generally, for the chelating agent to release the

25   paramagnetic substance. Preferably, therefore, the
complex contains two, one or zero open coordination
sites. The presence of more than two open sites in
general will unacceptably increase toxicity by release
of the metal ion in vivo.

30   In order to effectively enhance MRI images,
the complex is preferably capable of enhancing the
relaxation rates $1/T_1$ (longitudinal, or spin-lattice)
and/or $1/T_2$ (transverse, or spin-spin) of water protons
or other imaging or spectroscopic nuclei, including

protons, P-31, C-13, Na-23, or F-19 on other
biomolecules or injected biomarkers. Relaxivities R_1
and R_2 are defined as the ability to increase $1/T_1$ or
$1/T_2$, respectively, per mM of metal ion; units are

5 $mM^{-1}s^{-1}$. For the most common form of clinical MRI,
water proton MRI, relaxivity is optimal where the
paramagnetic ion bound to the chelating ligand still
has one or more open coordination sites for water
exchange. See S.M. Rocklage, et al. "Contrast Agents

10 in Magnetic Resonance", Magnetic Resonance Imaging,
Second Edition, Volume 1, Chapter 14, (1992), Mosby-
Year Book, Inc.; R.B. Lauffer, Chemical Reviews (1987),
87, pp. 901-927.

In addition to increasing the $1/T_1$ or $1/T_2$ of

15 tissue nuclei via dipole-dipole interactions, MRI
agents can affect two other magnetic properties and
thus be of use clinically:

1) an iron particle or metal chelate of high
magnetic susceptibility, particularly chelates of Dy,

20 Gd, or Ho, can alter the MRI signal intensity of tissue
by creating microscopic magnetic susceptibility
gradients. See A. Villringer et al., Magn. Reson. Med.
(1988), 6, pp. 164-174. No open coordination sites on
a chelate are required for this application.

25 2) an iron particle or metal chelate can also
be used to shift the resonance frequency of water
protons or other imaging or spectroscopic nuclei,
including protons, P-31, C-13, Na-23, or F-19 on the
injected agent or the protein to which it binds. Here,

30 depending on the nucleus and strategy used, zero to
three open coordination sites may be employed.

The preferred paramagnetic metal is selected
from the group consisting of Gd(III), Fe(III), Mn(II)
and Mn(III), Cr(III), Cu(II), Dy(III), Tb(III),

Ho(III), Er(III) and Eu(III). The most preferred is
Gd(III).

The organic chelating ligand should be
physiologically compatible. The molecular size of the
5 chelating ligand should be compatible with the size of
the paramagnetic metal. Thus gadolinium (III), which
has a crystal ionic radius of 0.938A, requires a larger
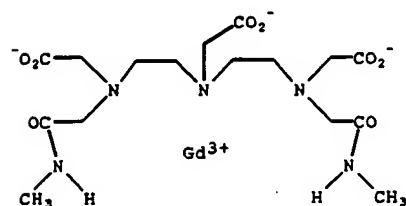chelating ligand than iron (III), which has a crystal
ionic radius of 0.64A.

10 Many suitable chelating ligands for MRI
agents are known in the art. These can also be used
for metal chelates for other forms of biological
imaging. For MRI imaging, preferred IEMs include:
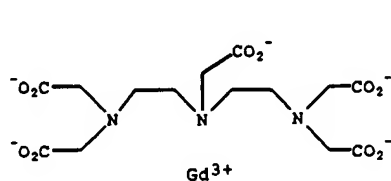
Magnevist
gadopentetate dimeglumine
DTPA
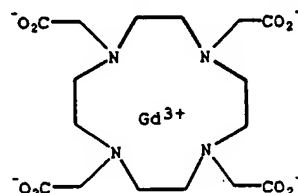
Dotarem
gadoterate meglumine
DOTA

Omniscan
gadodiamide
DTPA-BMA
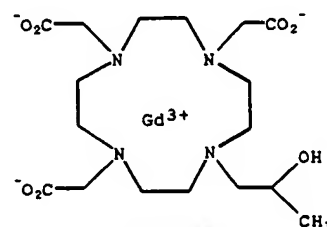
ProHance
gadoteridol
HP-DO3A

It is known in the art that other metals may
15 be substituted for Gd^{3+} in certain applications.

It is also contemplated that the IEM may
comprise a pharmaceutically acceptable salt.
Pharmaceutically acceptable salts of this invention
include those derived from inorganic or organic acids

and bases. Included among such acid salts are the
following: acetate, adipate, alginate, aspartate,
benzoate, benzenesulfonate, bisulfate, butyrate,
citrate, camphorate, camphorsulfonate, cyclopentane-
5   propionate, digluconate, dodecylsulfate,
ethanesulfonate, fumarate, glucoheptanoate,
glycerophosphate, hemisulfate, heptanoate, hexanoate,
hydrochloride, hydrobromide, hydroiodide, 2-hydroxy-
ethanesulfonate, lactate, maleate, methanesulfonate, 2-
10 naphthalenesulfonate, nicotinate, oxalate, pamoate,
pectinate, persulfate, 3-phenyl-propionate, picrate,
pivalate, propionate, succinate, tartrate, thiocyanate,
tosylate and undecanoate. Base salts include ammonium
salts, alkali metal salts, such as sodium and potassium
15 salts, alkaline earth metal salts, such as calcium,
magnesium and zinc salts, salts with organic bases,
such as dicyclohexylamine salts, N-methyl-D-glucamine,
and salts with amino acids such as arginine, lysine,
and so forth. Also, the basic nitrogen-containing
20 groups can be quaternized with such agents as lower
alkyl halides, such as methyl, ethyl, propyl, and butyl
chloride, bromides and iodides; dialkyl sulfates, such
as dimethyl, diethyl, dibutyl and diamyl sulfates, long
chain halides such as decyl, lauryl, myristyl and
25 stearyl chlorides, bromides and iodides, aralkyl
halides, such as benzyl and phenethyl bromides and
others. Water or oil-soluble or dispersible products
are thereby obtained. The preferred salts of the IEM
are the N-methyl-D-glucamine, calcium and sodium salts.

30   B.   Protein binding moiety (PBM)
        The PBM of the contrast agents of this
invention contribute to the binding of the agents to
one or more proteins within the tissue containing the

bioactivity. This non-covalent binding should be
sufficiently tight and the total number of binding
sites for the PBM sufficiently large such that contrast
is generated between tissues having different levels of
5   targeted bioactivity.

Examples of suitable PBMs include: drugs,
lipophilic or amphiphilic organic molecules,
porphyrins, receptor ligands, steroids, lipids,
hormones, peptides, proteins, oligonucleotides (DNA,
10   RNA or chemically modified versions thereof),
antibodies (including monoclonal and genetically
engineered versions and their fragments) or other
biomolecules or substances known to bind to one or more
proteins in the tissue containing the bioactivity
15   desired to be imaged.

Preferred PBMs are those that bind reversibly
to proteins in plasma, interstitial space (the fluid
between cells), or intracellular space. While any
biomolecule or substance that binds to a protein could
20   be used, most useful are those that bind to proteins
which either exist in high concentration or have a
large number of binding sites for the biomolecule or
substance. This affords the ability to temporarily
"trap" the large number of modified agents
25   catalytically produced by the bioactivity. The
reversible nature of the binding increases the
likelihood that the agents will eventually be excreted,
a very desirable property for imaging agents.

Examples of preferred proteins are: human
30   serum albumin (HSA) (0.7 mM in plasma, lower
concentrations in interstitial space); fatty acid
binding protein (FABP; also known as Z-protein or
protein A) (roughly 0.1 mM in the primary cells of the
liver, kidney, heart and other tissues); glutathione-S-

transferase (GST, also known as ligandin), (roughly 0.1 mM in the primary cells of the liver, kidney, heart and other tissues); alpha 1-acid glycoprotein (AAG, MW 41,000) (0.55g - 1.4g/L) and lipoproteins (concentrated in atherosclerotic plaque). Other preferred examples include the structural proteins of the extracellular matrix (collagens, laminin, elastin, fibronectin, entactin, vitronectin), amyloid (including the beta-2 amyloid protein (A4) of Alzheimer's disease), ceroid (or lipofuscin), and glycoproteins (for example, osteonectin, tenascin, and thrombospondin).

A more preferred protein for positively charged contrast agents or contrast agents containing basic PBMs is alpha 1-acid glycoprotein (AAG). The plasma levels of this positive acute phase protein varies significantly with disease state. For example, the concentrations of AAG increase two to four fold following inflammatory stimuli and plasma levels of AAG have been suggested as a prognostic aid for glioma, metastatic breast and other carcinoma, neonatal infection, and chronic pain. Elevated levels have been noted in atheroscerosis, Chron's disease, myocardial infarction, nephritis, and bacterial, viral, and post-operative infections. Ligands that bind AAG include numerous basic drugs, such as propranolol $(K_a = 11.3 \times 10^5)$, imipramine $(K_a = 2.4 \times 10^5)$, and chlorpromazine $(K_a = 35.4 \times 10^5)$, which can therefore be employed as PBMs.

Ligands for HSA, FABP, and GST are more preferred PBMs since these tend to be neutral with partial negatively charged groups (e.g., an ester, amide, or ketone carbonyl oxygen); such compounds are, in general, less toxic than positively charged molecules. For activated agents designed to bind FABP

or GST, hydrophobic long-chain PBMs which mimic the natural ligands (such as palmitic acid), or ring-containing PBMs (such as indocyanine green) are preferred.

5   Of these three proteins, HSA is highly preferred since ligands for HSA, unlike ligands for FABP and GST, require no intracellular uptake before binding. There need be no intracellular uptake of ligands for HSA since HSA is present in substantial

10 quantities in many extracellular fluid environments including plasma, interstitial space of normal and cancerous tissues, synovial fluid, cerebral spinal fluid and inflammatory or abscess fluid. In many pathologic tissues such as tumors, inflammation,

15 atherosclerotic plaque or the walls of atherosclerotic arteries, capillaries are leaky, resulting in even higher localized HSA levels.

        Another reason why HSA is highly preferred is that each protein molecule has a large number of ligand

20 binding sites. See U. Kragh-Hansen, Pharm. Rev. (1981), 33, pp. 17-53; X.M. He et al., Nature (1992), 358, pp. 209-215; D.C. Carter, Adv. Protein Chem. (1994), 45, pp. 153-203.

        The design of suitable PBMs is discussed in

25 U.S. patent no. 4,880,008 and in U.S. application Ser. No. 08/382,317 (filed February 1, 1995). For binding to HSA, a wide range of hydrophobic or amphiphilic substances function as the PBM. These include but are not limited to aliphatic, alkoxy or alkylthio,

30 alkylcarbonyl, alkylcarbonyloxy, aryl or heterocyclic groups with 1 to 60 carbons and, optionally, one or more nitrogen, oxygen, sulfur, halogen, aliphatic, amide, ester, sulfonamide, acyl, sulfonate, phosphate, hydroxyl or organometallic substituents.
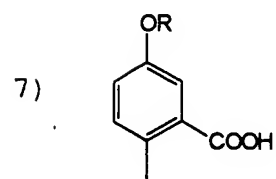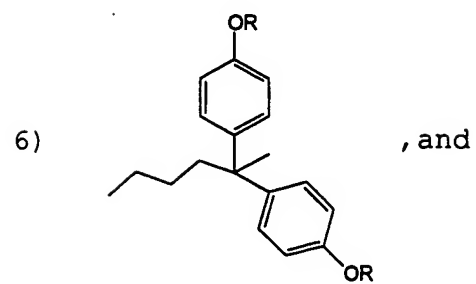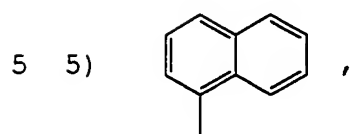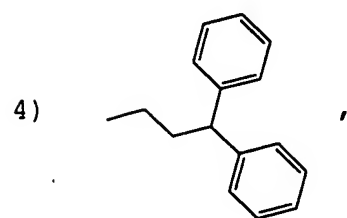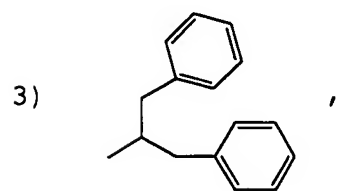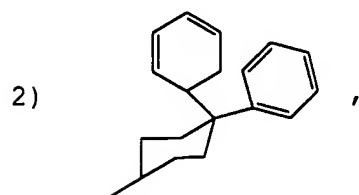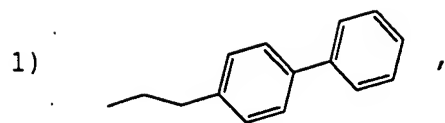
Alternatively, the PBM may be a peptide containing
hydrophobic amino acid residues and/or substituents
with or without hydrophobic or hydrophilic termination
groups.

5   The addition of lipophilic groups into a
contrast agent is likely to decrease the solubility of
the agent. To retain efficient solubility of the
contrast agent at clinically effective dosage levels or
higher, it may be preferred to incorporate one or more
10 hydrogen-bonding groups (oxygen, nitrogens, etc.) into
the PBM.

While purely aliphatic groups can be used as
PBMs, these may not be as preferred as mixed aliphatic-
aryl groups or purely aryl groups. Especially when a
15 negative charge is attached to the terminus of long and
flexible aliphatic groups, the contrast agents tend to
disrupt the interactions between membrane proteins and
lipids. This may increase the toxicity of the agent.
Thus it is preferred that the PBM contain at least one
20 aryl ring.

In the case of HSA-bound MRI agents for
tissue enhancement, it is preferable for the contrast
agent to contain two or more lipophilic groups to fully
immobilize the agent when bound to the protein. These
25 groups may be on one PBM, or as two or more separate
chemical groups attached to the contrast agent.
Because of their bulky nature and rigidity, it is
highly preferable that the two or more groups each
contain an aryl ring, with the two or more rings
30 arranged in a rigid, non-planar orientation.

Preferred PBMs suitable for incorporation
into the prodrug contrast agents of this invention
include the following structures:

1) ,

2) ,

3) ,

4) ,

5 5) ,

6) ,and

7)

wherein R comprises an aliphatic group and/or at least one aryl ring, or comprises a peptide containing hydrophobic amino acid residues and/or substituents with or without hydrophobic or hydrophilic
5 termination groups.

The magnetic resonance phenomena is complex, and different paramagnetic materials alter the MRI signal to various degrees. See R.B. Lauffer, _Chemical Reviews_ (1987), 87, pp. 901-927. A quantitative
10 measurement of the ability of a contrast agent to relax water protons, and consequently affect the MRI image, is provided by its relaxivity. Relaxivity is the dependence of water proton signal intensity upon the concentration of paramagnetic metal ion in solution.
15 Relaxivity is defined as the induced T_1 or T_2 relaxation per unit time (R_1 or R_2 in units of $mM^{-1} sec^{-1}$) observed for a contrast agent, where the concentration of the agent is expressed in a millimolar (mM).

The physical properties of a gadolinium
20 complex affect the relaxivity of the contrast agent. The number of water molecules bound to the gadolinium complex, the rate of exchange of the water molecule with bulk solution, the relaxation time of the seven unpaired electrons, and the rotational tumbling time
25 (know as the rotational correlation time) of the contrast agent in solution all contribute to the overall observed relaxivity. Alteration in these physical properties can dramatically alter the relaxivity. For example, the binding of small-
30 molecular-weight gadolinium chelates to large macromolecules slows the rotation tumbling time and increases the relaxation enhancement by factors of 3 to 10. Binding of the contrast agent to the protein causes the magnetic fluctuations between the

paramagnetic ion and the water protons to occur on the
same time scale as the Larmor frequency, generating the
most efficient longitudinal (T_1) relaxation possible
and the highest possible relaxivity. Thus, non-

5 covalent binding of MRI contrast agents to large
macromolecules, such as proteins, is an efficient way
to increase the MRI signal. Image contrast is
generated between areas which have different levels of
non-covalent binding of an activated agent.

10   To generate bioactivity-related contrast
between normal and abnormal tissues, there should be a
substantial difference between the protein-binding
affinity of the prodrug and that of the bioactivated
agent. The prodrug binding affinity is desirably 80%

15 or less of the binding affinity of the activated agent.
For example, if the activated agent is 90% bound to a
protein within a tissue containing the bioactivity
under physiologically relevant conditions (i.e.,
contrast agent concentration in plasma 0.01-10mM for

20 MRI and optical imaging), the prodrug should be 72%
bound or less under the same conditions. It is
preferred that the prodrug exhibit 50% or less of the
binding affinity of the activated agent, more preferred
is 40% or less, even more preferred is 30% or less,

25 even more preferred is 20% or less, and most preferred
is 10% or less.

         In MRI, the bioactivity-related contrast
between normal and abnormal tissues can be manifested
as a change in the induced relaxation rates ($1/T_1$ or

30 $1/T_2$) of water protons, or relaxivities, R_1 and R_2. In
the present invention, the prodrug relaxivity R_1 is
desirably 80% or less of the R_1 of the bioactivated
agent. Preferably the prodrug relaxivity R_1 is 50% or
less of the relaxivity R_1 of the bioactivated agent,

more preferably 20% or less, and most preferably 10% or less.

Protein binding of contrast agents can be assessed in vitro by equilibrium dialysis or
5 ultrafiltration using a physiologically relevant protein concentration in buffer. See, G.N. Rolinson and R. Sutherland, British J. Pharmac. Chemother. (1965), 25, p. 638 (ultrafiltration); D. Glick , Methods Biochem. Anal. (1956), 3 p. 265 (equilibrium
10 dialysis). The protein binding measurements set forth in this application are determined by ultrafiltration using 4.5%(w/w) human serum albumin in phosphate buffered saline (0.15 NaCl, 10mM phosphate, pH 7.4). Preferably at least 10%, and more preferably at least
15 50%, and even more preferably at least 80%, and most preferably at least 95%, of an activated protein-binding contrast agent will be bound to the protein under physiologically relevant conditions. In this application, the measurement of percent binding of the
20 contrast agent to HSA has an error of approximately +/- 5%. Protein binding to other proteins or to serum can be assessed in a similar fashion.

The degree to which an agent has been tuned for maximum relaxivity can be assessed by measuring the
25 relaxivity-bound (R_1-bound) in the presence of HSA. This requires measuring the relaxivity of the free chelate (R_1-free) as well as the relaxivity (R_1-observed) and per cent binding of the agent in 4.5% HSA. The R_1-observed is a mole fraction weighted
30 average of R_1-free and R_1-bound:

R1-observed = (fraction-free * R_1-free) +
                (fraction-bound * R_1-bound)

Thus:

$$R1\text{-bound} = \frac{[R1\text{-observed} - (\text{fraction-free} * R1\text{-free})]}{\text{fraction-bound}}$$

Protein binding of ligands can also be
5 evaluated theoretically. For a common class of
ligands, binding affinity to HSA and other proteins
will generally increase with the hydrophobicity of the
PBM. Theoretical estimates of the hydrophobicity of a
substituent such as a PBM can be obtained by
10 calculating the contribution to the log of the octanol-
water (or octanol-buffer) partition coefficient (log P)
for the PBM itself using the Hansch π constant for
substituents. See A. Leo and C. Hansch, "Partition
Coefficients and their Uses," Chemical Reviews, 71,
15 pp. 525-616 (1971); K.C. Chu, "The Quantitative
Analysis of Structure-Activity Relationships," Burger's
Medicinal Chemistry, Part 1, pp. 393-418, (4th ed.
1980). Binding affinity will increase with increasing
log P contributions. For example, for substituents on
20 aliphatic groups, the following π constants can be
used:

Group	π-aliphatic
CH_3	0.50
Phenyl	2.15

25 For substituents on aryl groups, the following [1]
constants can be used:

Group	π-aliphatic
CH_3	0.56
CH_2CH_3	1.02

Phenyl   1.96

$C(CH_3)_3$ 1.98

Thus, the log P contribution for a p-methylbenzyl group attached to an IEM would be calculated as follows (using the value of the [1]-aliphatic for CH_3 as an estimate for the $-CH_2-$ group):

log P contribution = 0.50 + 2.15 + 0.56 = 3.21

The log P contribution for a p-[4-(t-butyl)-phenyl]benzyl group attached to an IEM would be calculated as follows (using the value of the π-aliphatic for CH_3 as an estimate for the $-CH_2-$ group):

log P contribution = 0.56 + 2.15 + 2.15 + 1.98 = 6.84

In binding to HSA, a minimum log P contribution of 2 (equivalent to four CH_3 groups or one phenyl ring) is required to achieve significant binding. More preferred is a log P contribution of 4 or more. Even more preferred is a log P contribution of 6 or more.

In optical imaging, the invention requires that there be a measurable difference between the optical properties of the non-protein bound prodrug, and the bioactivated protein bound contrast agent. For example, the maximal absorbance of indocyanine green is shifted from 770-780 to 790-805 nm upon binding to proteins in plasma or blood. This difference is used to detect bioactivity by imaging or detecting the protein-bound, activated optical imaging agent. As in the case of MRI, use of a bioactivated prodrug of the optical agent increases the specificity of the agent.

C.   Modification Site (MS)

The Modification Site (MS) domain on the
prodrug is altered by the specific bioactivity desired
to be imaged. That alteration, which is a
5 biotransformation (enzymatic or otherwise) such as bond
cleavage, bond formation, oxidation, reduction, or
protonation/deprotonation, results in the generation of
bioactivated agent. The MS can be an inherent part of
the IEM or PBM (as long as it does not adversely affect
10 their individual functions) or it can constitute a
separate substituent. One skilled in the art will
recognize the chemical structures of the MS which are
capable of being altered by the bioactivity.

Preferred MSs are those capable of being
15 altered in vivo by enzymes. Enzymes useful to modify
the prodrugs of this invention are those expressed in
mammals or in infectious microorganisms (bacteria,
yeast, viruses, etc.) which promote modification or
cleavage of one or more bonds in the prodrug. The
20 expression of enzyme molecules and their associated in
vivo inhibitors is very sensitive to the type of tissue
or its condition. Highly preferred modification sites
are those which are altered by enzymes which have
elevated levels or activity in patients who have
25 inflammatory diseases, infectious disease, cancer,
atherosclerosis, thrombosis, myocardial infarction,
rheumatoid arthritis, osteoarthritis, endometriosis,
periodontal disease, autoimmune disease, and so forth.
In the case of enzymatic bioactivity, the MS chemical
30 structure will be closely related to that of the
natural or optimal substrates for the enzyme. The
natural or optimal substrates are well-known and

described in the literature or can be identified by
standard biochemical techniques.

      Preferred modification sites include those
which are cleaved by the EC class of enzymes known as
5 Hydrolases (EC 3.1.*.* through EC 3.99.*.*). These
modification sites consist of carbon-oxygen, carbon-
nitrogen, phosphorous-oxygen, carbon-carbon and other
bonds which are hydrolytically cleaved by the action of
the appropriate enzyme. More preferred modification
10 sites include phosphorous-oxygen bonds, which are
hydrolysed by enzymes known as phosphatases
(EC.3.1.3.*) (Class, Hydrolase; subclass, esterase;
sub-subclass, phosphomonoesterase). Specific examples
of phosphatase enzymes and their common names are
15 listed in Table I below.

<div align="center">TABLE I</div>

EC Number	Common Name	Other Names
EC 3.1.3.1	Alkaline Phosphatase	Alkaline phospho-monoesterase; phosphomono-esterase; glycero-phosphatase
EC 3.1.3.2	Acid Phosphatase	Acid phosphomono-esterase; phosphomono-esterase; glycero-phosphatase

(line numbers: 20 aligns with Alkaline row, 25 with Acid row, 30 with glycero-phosphatase)

      A specific example of a phosphorous-oxygen MS
site is that contained in prodrug MRI contrast agent 1.
Such phosphate mono-ester derivatives are rapidly
35 hydrolyzed by alkaline phosphatase to generate an

alcohol (or phenol) and phosphate (PO_4^{2-}) as products. In this specific case, the phosphate mono-ester prodrug 1 binds HSA less strongly than its enzymatic cleavage product, the corresponding alcohol. The clinical

5 relevance of enzymes which act on phosphorous-oxygen MS sites is exemplified by the case of acid phosphatase, which has elevated levels in prostate cancer patients and has been used extensively in the diagnosis, staging and monitoring of prostate cancer for decades.

1
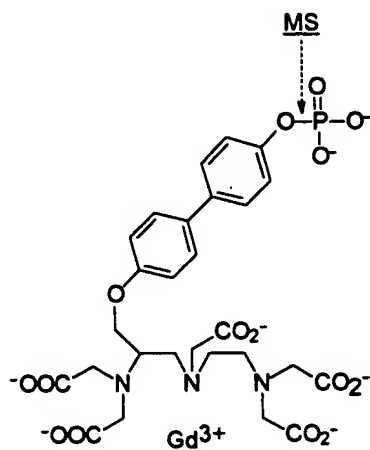
10   Additional preferred modification sites include those which are cleaved by sulfatases (EC 3.1.6.*; Class, Hydrolase; subclass, esterase; sub-subclass, sulfatase), enzymes which cleave sulfur-oxygen bonds. Steroid sulfatase activity is

15 particularly high in breast tumors, and plays a role in regulating the formation of estrogens within tumors. A listing of sulfatases able to alter sulfate MS sites and their EC numbers are listed in Table II below.

TABLE II

EC Number	Common Name	Other Names
EC 2.8.2.4	Estrone Sulfatase	Estrone Sulfo-trans-ferase
EC 2.8.2.15	Steroid sulfotrans-ferase	
EC 3.1.6.2	Steryl-sulfatase	
EC 3.1.5.1	Arylsulfatase	Sulfatase
EC 3.1.6.4	N-acetylgalactosamine-6-sulfatase	
EC 3.1.6.11	Disulfoglucosamine-6-sulfatase	
EC 3.1.6.18	Glucuronate-2-sulfatase	
EC 3.1.6.6	Choline-sulfatase	
EC 3.1.6.8	Cerebroside-sulfatse	
EC 3.1.6.9	Chondro-4-sulfatase	
EC 3.1.6.10	Chondro-6-sulfatase	
EC 3.1.6.12	N-acetylgalactosamine-4-sulfatase	
EC 3.1.6.13	Iduronate-2-sulfatase	
EC 3.1.6.16	Monomethyl-sulfatase	
EC 3.1.6.17	D-lactate-2-sulfatase	

Highly preferred MSs are carbon-nitrogen peptide bonds which are hydrolyzed by a subclass of hydrolase enzymes known as proteinases (EC 3.4.*.*). These enzymes hydrolyze an amide bond to form two cleavage products, an amine and a carboxylic acid, one of which remains attached to the bioactivated agent.

The MS is designed to mimic a natural or optimal
peptide substrate for the specific enzymatic activity
to be imaged.

Especially preferred carbon-nitrogen
5   peptide MSs are those which are hydrolyzed by serine
proteases (EC 3.4.21.*; Class, Hydrolase; subclass,
peptidase, sub-subclass, serine endopeptidase). Serine
protease activity has been linked to primary breast
cancer, tumor progression that leads to metastasis in
10   breast cancer, the activation of coagulation in
patients with lung cancer, pancreatic cancer, severe
pancreatitis, and prostate cancer. An MS useful for
diagnostic agents for prostate cancer is one which is
altered by prostate-specific antigen (PSA), a serine
15   protease glycoprotein (30-34 kDa) produced exclusively
by prostatic tissue. PSA exhibits enzymatic activity
typical of peptidases chymotrypsin and trypsin, and its
physiological substrate appears to be high-molecular-
mass seminal vesicle protein (HMM-SV-protein). PSA is
20   extremely useful for monitoring therapy, particularly
prostatectomy because its presence is decreased to
nearly zero following removal of the prostate. A slow
rise in PSA following prostatectomy indicates that
either not all of the prostate is removed or that lymph
25   node metastases are present and producing the antigen.
The concentration of PSA is also proportional to tumor
burden or malignant potential and changes quickly in
response to therapy. A listing of specific serine
proteases enzymes and their common names are listed in
30   Table III below.

TABLE III

EC Number	Common Name	Other Names
EC 3.4.21.77	Prostate-specific antigen	Semenogelase; PSA; gamma-seminoprotein seminin
EC 3.4.21.37	Leukocyte Elastase	Lysosomal elastase; Neutrophil; elastase; Bone marrow serine protease; Medullasin;
EC 3.4.21.36	Pancreatic Elastase	Pancreato-peptidase E; Pancreatic elastase I
EC 3.4.21.76	Myeloblastin	Proteinase 3 Wegener's autoantigen

Preferred MSs are those which are altered by matrix metalloproteinases (MMPs) (EC 3.4.24.*, subclass, peptidase; sub-subclass metalloendopeptidase), enzymes which exhibit high bioactivity in the extracellular space, a tissue compartment which is easily accessible to contrast agents. Furthermore, MMP activity is altered by many diseases. To varying degrees, members of the MMP family are linked to the following diseases: cancer (especially in the degradation of extracellular matrix prior to metastases), atherosclerosis (especially in the degradation of the fibrous cap of atherosclerotic plaque leading to rupture, thrombosis, and myocardial infarction or unstable angina), rheumatoid arthritis and osteoarthritis (destruction of cartilage aggrecan

and collagen), periodontal disease, inflammation,
autoimmune disease, organ transplant rejection,
ulcerations (corneal, epidermal, and gastric),
scleroderma, epidermolysis bullosa, endometriosis,
5   kidney disease, and bone disease. Specific
metalloproteinase enzymes and their common names are
listed in Table IV below.

TABLE IV

EC Number	Common Name	Other Names
10 EC 3.4.24.23	Matrilysin	MMP-7; Matrin; Uterine metallo- endopeptidase; PUMP-1
15 EC 3.4.24.7	Interstitial collagenase ·	MMP-1; Vertebrate collagenase; Fibroblast collagenase
20 EC 3.4.24.17	Stromelysin-1	MMP-3; Transin; Proteoglycanase
EC 3.4.24.22	Stromelysin-2	MMP-10; Transin-2
25 EC 3.4.24.24	Gelatinase	MMP-2; 72-kDa gelatinase; Type IV collagenase;
EC 3.4.24.26 30	Pseudolysin	Pseudomonas in elastase; Pseudomonas aeruginosa-neutral metalloproteinase
EC 3.4.24.34 35	Neutrophil collagenase	MMP-8

EC 3.4.24.35	Gelatinase B	MMP-9; 92-kDa gelatinase; Type V collagenase; 92-kDa type IV-collagenase; Macrophage gelatinase
EC 3.4.24.39	Deuterolysin	Penicillium Rogqueforti-protease II; Microbial neutral-proteinase II; Acid metalloproteinase

In the case where the targeted bioactivity is the enzymatic activity expressed by MMP-1, a matrix metalloproteinase which is elevated in certain inflammatory diseases, a preferred MS is the carbon-nitrogen amide bond linking the amino acids glycine (Gly) and isoleucine (Ile). An example of a prodrug containing a Gly-Ile amide bond MS site is prodrug compound 2.
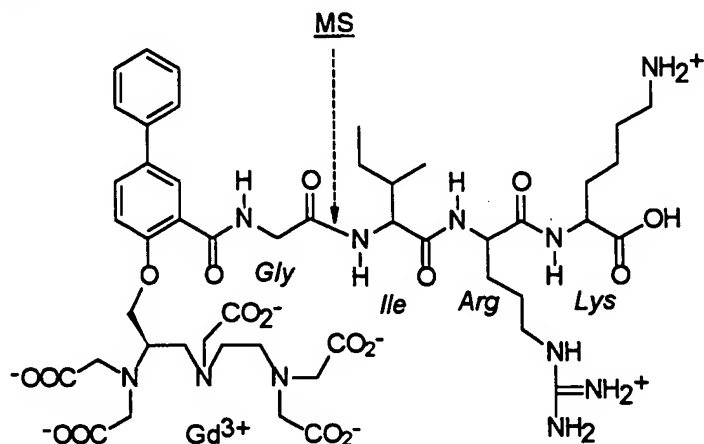
It will be apparent to those skilled in the art that other types of MS (for example, esters, ethers) are hydrolyzed by appropriate target enzymes,

such as those categorized as esterases (EC 3.1.*.*) or
ether hydrolases (EC 3.3.*.*) and that, based on the
knowledge of the chemistry of the target enzyme,
optimal MSs may then be incorporated into the prodrug.

5   In some cases, it is desirable that
alteration of the modification site be followed by a
second, chemical reaction in order to generate the
activated contrast agent. Neutral or negatively
charged PBMs are preferred over positively charged PBMs
10 for those agents which are designed to bind HSA (see
U.S. patent no. 4,880,008). Thus, an especially
preferred method for activation of contrast agents that
bind to HSA is a secondary chemical reaction which
converts positively charged MS cleavage residue to
15 neutral or negatively charged group. This increases
the hydrophobicity of the agent (increased log P) and
tends to increase HSA binding.

        D.   Masking Moiety (MM)

        When present in a prodrug of this invention,
20 an MM is cleaved from the prodrug when it is
bioactivated. An MM can be any organic or inorganic
moiety which, when incorporated into the prodrug in a
proper position, decreases the protein binding affinity
of the prodrug compared to the bioactivated contrast
25 agent.
        Examples of suitable MMs include
polyethylenegylcol, dextran, hyaluronic acid, or other
substances that alter the charge or hydrophobicity of
the surface of the PBM. Such materials have been
30 widely used to prevent the interaction large molecular-
weight materials (for example, polymers, proteins, or
liposomes) with cellular surfaces in the blood. For
example, polyethyleneglycol (PEG) attached to liposomes

prevents cellular uptake into the reticuloendothelial
system, resulting in prolonged circulation of the
liposomes. See D. Paphadjopoulos et al., _Proceedings_
of the National Academy of Sciences (1991), _88_,
5   pp. 11460-11464; T.M. Allen et al., _Biochimica_
Biophysica Acta (1991), _1066_, pp. 29-36.

   For low molecular weight (<5000 Daltons)
prodrug contrast agents, hydrophilic and/or charged
groups can similarly be used. Such groups can be
10 judiciously positioned within the MM/Linker so that
they effectively mask protein binding, yet are released
upon bioactivation, thus allowing the increased binding
capability of the IEM/PBM to be expressed. For
contrast agents which are designed to bind serum
15 proteins such as HSA following bioactivation,
hydrophilic and/or charged groups such as hydroxyl,
amine (or ammonium), quaternary amine, certain amino
acids (especially lysine, arginine, and histidine),
sulfoxide, phosphate, sulfate, carboxylate,
20 carbohydrate, sugar, and metal chelates in single or
multiple configurations represent potentially effective
MMs.

   Examples of hydrophilic and/or charged groups
which affect HSA binding affinity are described in the
25 art. HSA binding of iodinated x-ray contrast agents is
masked by the judicious substitution of hydroxyl groups
combined with the elimination of carboxylate groups.
For example, the X-ray contrast agent iodipamide binds
to HSA with high affinity (>98%), whereas corresponding
30 neutral iodinated x-ray contrast agents, which are
modified to contain numerous hydrophilic hydroxyl
groups, bind to HSA with low affinity (<1%). See
Radiocontrast Agents, M. Sovak, ed., Springer-Verlag,
New York (1984), Chapter 1 "Chemistry of X-Ray Contrast

Media," pp.23-125. Similarly, a reduction of HSA binding affinity for a series of bile acid derivatives is noted as the number of hydrophilic hydroxyl groups is increased. Thus, lithocholic acid (binding

5 constant = 2.0×10^5) binds more tightly than chenodeoxycholic acid cholic and (binding constant = 5.5×10^4) which binds more tightly than cholic acid (binding constant = 0.3×10^4). See Roda et al., J. Lipid Research (1982), 23, pp. 490-495.

10   Appropriately positioned primary, secondary or tertiary amines have been shown to reduce the HSA binding affinity of certain antibiotics as compared with similar drugs lacking this functionality. This effect is illustrated by data reported for some novel

15 antibiotics, enoxacin and NY-198. See E. Okezaki et al., Drug Metabolism and Disposition (1988), 16, pp. 865-74. The fraction of these compounds which were bound to HSA (f_b) was reported to be 0.35 and 0.28, respectively, as compared with analogs miloxacin

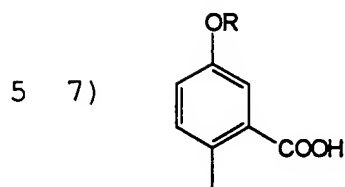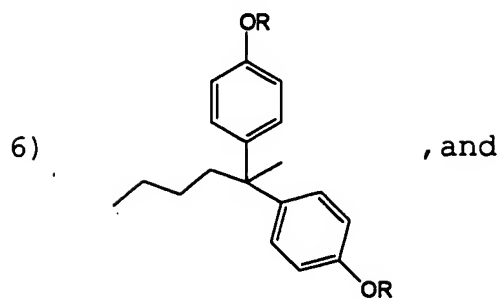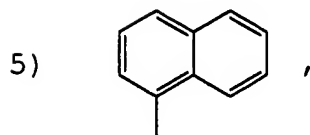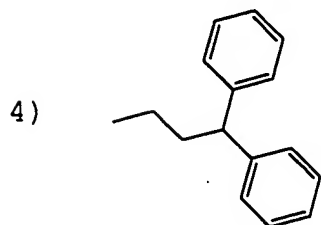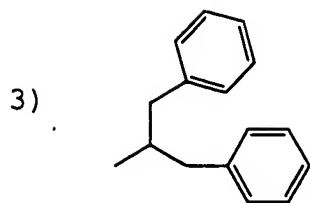20 (f_b=0.86) and nalidixic acid (f_b=0.71) which lacked the amine groups.

      Thus, in the preferred prodrugs of this invention, the IEM comprises a DTPA, DOTA, DTPA-BMA or HP-DO3A chelate of Gd^{3+}; the PBM comprises one or more

25 of the following structures:
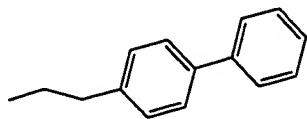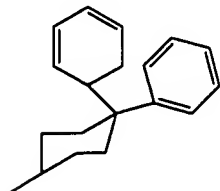
1)   ,

2)   ,

3) ,

4) ,

5) ,

6) , and

5 7)

wherein R comprises an aliphatic group and/or at least one aryl ring, or comprises a peptide containing hydrophobic amino acid residues and/or substituents with or without hydrophobic or hydrophilic

10 termination groups; and

the MS comprises a bond capable of being altered _in vivo_ by a hydrolase enzyme.

Preferably, the MS is a phosphorus-oxygen
bond capable of being hydrolyzed in vivo by a
phosphatase enzyme or an amide bond capable of being
hydrolyzed in vivo by metalloproteinase enzyme or a
5   serine protease enzyme. Gadolinium complex 1,
gadolinium complex 2 and gadolinium complex 10,
identified herin, are examples of preferred prodrugs.

III. Synthesis

The compounds of this invention may be
10 synthesized using conventional techniques.
Advantageously, these compounds are conveniently
synthesized from readily available starting materials.
Many starting materials are commercially available,
e.g., from Aldrich Chemical Company, Inc., Milwaukee,
15 WI. Although methods for the syntheses of the
compounds of this invention are known to those of
ordinary skill in the art of organic synthesis, the
following general methods are set forth to illustrate
these syntheses. These methods should not be viewed as
20 limiting the scope of this invention in any way.
The synthesis of DTPA and DOTA chelating
agents modified with organic substituents used to link
the resulting chelate covalently to proteins, including
monoclonal antibodies, has been described in the
25 literature. For example, 1-(p-
isothiocyanatobenzyl)DTPA was prepared by reacting
commercially available p-nitrophenylalanine methyl
ester (Aldrich Chemical) with ethylene diamine and
subsequent reduction with borane to form the triamine.
30 Alkylation with bromoacetic acid followed by reduction
(H_2/Pd-C) and reaction with thiophosgene gives the
isothiocyanate. See M.W. Brechbiel et al., Inorganic
Chemistry (1986), 25, pp. 2772-2781. Chelating agents

in which the DTPA carbon backbone has been substituted
with a aminobutyl group derived from derived from
lysine have also been reported. See J.P.L. Cox et al.,
<u>J. Chemical Society Perkin Transactions</u> I (1990),

5 pp. 2567-2576. A synthetic approach to acetate-
substituted DTPA molecules via double alkylation of p-
nitrophenylalanine has also been described. See M.A.
Williams et al., <u>Journal of Organic Chemistry</u> (1993),
<u>58</u>, pp. 1151-1158. Similarly, functionalized

10 macrocyclic DOTA molecules have been prepared starting
from amino acids and standard cyclization techniques,
including Richman-Atkins tosylate chemistry (J.P.L. Cox
et al., <u>J. Chemical Society Perkin Transactions I</u>
(1990), pp. 2567-2576) or high-dilution ring formation

15 (T.J. McMurry et al., <u>Bioconjugate Chemistry</u> (1992),
pp. 108-117.

The synthesis of hepatobiliary MRI contrast
agents containing lipophilic benzyl substituents is
described in U.S. patent no. 4,899,755. MRI contrast

20 agents containing PBMs and blood half-life extending
moieties are described in U.S. application ser. no.
08/382,317 (filed February 1, 1995). That application
describes the synthesis of DTPA chelating agents linked
to PBMs through phosphodiester linkages as well as the

25 synthesis of some versatile intermediates, including
carbonate hydroxymethyldiethylenetriamine, compound <u>3</u>,
and 1-hydroxymethyl-DTPA-penta-t-butyl ester, compound
<u>4</u> (Scheme 1).

Other versatile synthetic intermediates for

30 the preparation of prodrug contrast agents include 1-p-
hydroxybenzyl-diethylenetriamine (compound <u>6</u>). See
Schumhmann-Giampieri, <u>G. Radiology</u> (1992), <u>183</u>, pp. 59-
64. Compound <u>6</u> is converted to 1-(p-hydroxybenzyl)-

DTPA-penta-t-butyl ester, compound 7, by alkylation with t-butylbromoacetate (Scheme 1).

Scheme 1: Synthetic Intermediates
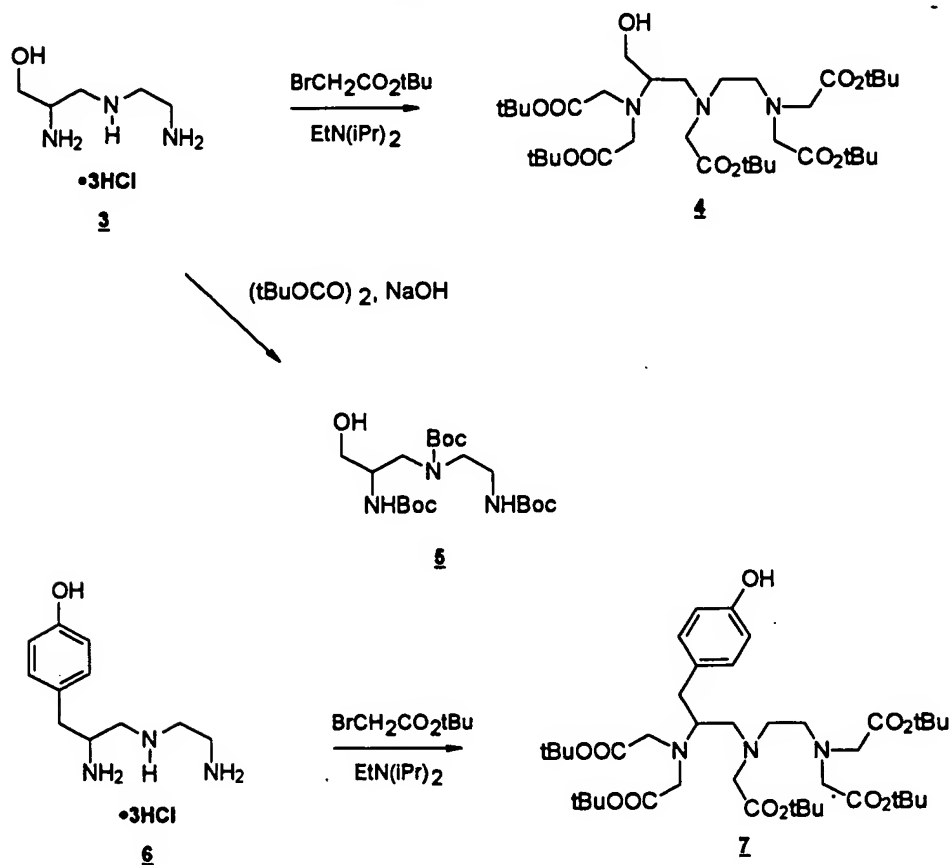
The carbamate 5 or penta-t-butyl ester
5 intermediates 4 and 7 are derivatized in a single step
with PBM groups which incorporate the desired
functional domains (MS, MM, L) as well as functional
groups which are known in the art to form covalent
bonds with hydroxyl or phenol groups (for example,
10 ethers are formed by reaction of alkyl halides with
alcohols or by diethyldiazodicarboxylate (DEAD)
catalyzed reaction with a second alcohol. See
J. March, Advanced Organic Chemistry, Third Edition
John Wiley & Sons (1995), p.1161 for other appropriate

reactions and covalent linkages. Alternatively, the MS
and optional MM and/or L domains are added to the PBM
in a stepwise fashion. For example, a PBM containing a
reactive alkyl halide and a second suitably protected
5 reactive group (e.g., hydroxyl or carboxylate) is -
coupled to the DTPA-penta-t-butyl ester via formation
of an ether linkage. Transient protection of reactive
groups may be accomplished by means known in the art.
See, e.g., Greene, T.W. and Wuts, P.G.M., Protective
10 Groups in Organic Synthesis, Second Edition ©1991 John
Wiley and Sons, Inc., New York, NY at pp. 10-276. The
second reactive group is then deprotected and modified
to add the desired MS or both an MS and MM. Final
deprotection of the t-butyl ester protecting groups
15 using acid (HCl or TFA) results in the penta acid free
ligand, which is then reacted with gadolinium(III) and
base to form the gadolinium complex.

As can be appreciated by the skilled artisan,
the above synthetic schemes are not intended to
20 comprise a comprehensive list of all means by which the
compounds described and claimed in this application may
be synthesized. Further methods will be evident to
those of ordinary skill in the art.

IV. Use of the Improved Contrast Agents

25 It is contemplated that pharmaceutical
compositions may be prepared comprising any of the
prodrugs of the present invention, or pharmaceutically
acceptable salts thereof, together with any
pharmaceutically acceptable carrier, adjuvant or
30 vehicle. The term "pharmaceutically acceptable
carrier, adjuvant or vehicle" refers to a carrier or
adjuvant that may be administered to a patient,
together with a compound of this invention, and which

does not destroy the activity thereof and is nontoxic
when administered in doses sufficient to deliver an
effective amount of the agent. Pharmaceutically
acceptable carriers, adjuvants and vehicles that may be
5 used in the pharmaceutical compositions of this
invention include, but are not limited to, ion
exchangers, alumina, aluminum stearate, lecithin, serum
proteins, such as human serum albumin, buffer
substances such as phosphates, glycine, sorbic acid,
10 potassium sorbate, TRIS (tris(hydroxymethyl)amino-
methane), partial glyceride mixtures of saturated
vegetable fatty acids, water, salts or electrolytes,
such as protamine sulfate, disodium hydrogen phosphate,
potassium hydrogen phosphate, sodium chloride, zinc
15 salts, colloidal silica, magnesium trisilicate,
polyvinyl pyrrolidone, cellulose-based substances,
polyethylene glycol, sodium carboxymethylcellulose,
polyacrylates, waxes, polyethylene-polyoxypropylene-
block polymers, polyethylene glycol and wool fat.
20   According to this invention, the
pharmaceutical compositions may be in the form of a
sterile injectable preparation, for example a sterile
injectable aqueous or oleaginous suspension. This
suspension may be formulated according to techniques
25 known in the art using suitable dispersing or wetting
agents and suspending agents. The sterile injectable
preparation may also be a sterile injectable solution
or suspension in a non-toxic parenterally-acceptable
diluent or solvent, for example as a solution in 1,3-
30 butanediol. Among the acceptable vehicles and solvents
that may be employed are water, Ringer's solution and
isotonic sodium chloride solution. In addition,
sterile, fixed oils are conventionally employed as a
solvent or suspending medium. For this purpose, any

bland fixed oil may be employed including synthetic
mono- or di-glycerides. Fatty acids, such as oleic
acid and its glyceride derivatives are useful in the
preparation of injectables, as are natural pharmaceuti-
5   cally-acceptable oils, such as olive oil or castor oil,
especially in their polyoxyethylated versions. These
oil solutions or suspensions may also contain a long-
chain alcohol diluent or dispersant.

In some cases, depending on the dose and rate
10 of injection, the binding sites on plasma proteins may
become saturated with prodrug and activated agent.
This leads to a decreased fraction of protein-bound
agent and could compromise its half-life or
tolerability as well as the effectiveness of the agent.
15 In these circumstances, it is desirable to inject the
prodrug agent in conjunction with a sterile albumin or
plasma replacement solution. Alternatively, an
apparatus/syringe can be used that contains the
contrast agent and mixes it with blood drawn up into
20 the syringe; this is then re-injected into the patient.

The prodrug compounds and pharmaceutical
compositions of the present invention may be
administered orally, parenterally, by inhalation spray,
topically, rectally, nasally, buccally, vaginally or
25 via an implanted reservoir in dosage formulations
containing conventional non-toxic pharmaceutically-
acceptable carriers, adjuvants and vehicles. The term
"parenteral" as used herein includes subcutaneous,
intravenous, intramuscular, intra-articular, intra-
30 synovial, intrasternal, intrathecal, intrahepatic,
intralesional and intracranial injection or infusion
techniques.

When administered orally, the pharmaceutical
compositions of this invention may be administered in

any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and

5 corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are required for oral use, the active

10 ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

Alternatively, when administered in the form of suppositories for rectal administration, the

15 pharmaceutical compositions of this invention may be prepared by mixing the agent with a suitable non-irritating excipient which is solid at room temperature but liquid at rectal temperature and therefore will melt in the rectum to release the drug. Such materials

20 include cocoa butter, beeswax and polyethylene glycols.

As noted before, the pharmaceutical compositions of this invention may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical

25 application, including the eye, the skin, or the lower intestinal tract. Suitable topical formulations are readily prepared for each of these areas or organs.

Topical application for the lower intestinal tract can be effected in a rectal suppository

30 formulation (see above) or in a suitable enema formulation. Topically-transdermal patches may also be used.

For topical applications, the pharmaceutical compositions may be formulated in a suitable ointment

containing the active component suspended or dissolved
in one or more carriers. Carriers for topical
administration of the compounds of this invention
include, but are not limited to, mineral oil, liquid

5   petrolatum, white petrolatum, propylene glycol, poly-
oxyethylene, polyoxypropylene compound, emulsifying wax
and water. Alternatively, the pharmaceutical composi-
tions can be formulated in a suitable lotion or cream
containing the active components suspended or dissolved

10   in one or more pharmaceutically acceptable carriers.
Suitable carriers include, but are not limited to,
mineral oil, sorbitan monostearate, polysorbate 60,
cetyl esters wax, cetearyl alcohol, 2-octyldodecanol,
benzyl alcohol and water.

15   For ophthalmic use, the pharmaceutical
compositions may be formulated as micronized
suspensions in isotonic, pH adjusted sterile saline,
or, preferably, as solutions in isotonic, pH adjusted
sterile saline, either with our without a preservative

20   such as benzylalkonium chloride. Alternatively, for
ophthalmic uses, the pharmaceutical compositions may be
formulated in an ointment such as petrolatum.
              For administration by nasal aerosol or
inhalation, the pharmaceutical compositions of this

25   invention are prepared according to techniques well-
known in the art of pharmaceutical formulation and may
be prepared as solutions in saline, employing benzyl
alcohol or other suitable preservatives, absorption
promoters to enhance bioavailability, fluorocarbons,

30   and/or other conventional solubilizing or dispersing
agents.
              The amount of active ingredient that may be
combined with the carrier materials to produce a single
dosage form will vary depending upon the host treated

and the particular mode of administration. A typical preparation will contain from about 5% to about 95% active compound (w/w). Preferably, such preparations contain from about 20% to about 80% active compound.

5   For intravenous and other types of administration, acceptable dose ranges are between 0.001 and 1.0 mmol/kg of body weight, with the preferred dose of the active ingredient compound being between 0.001 and 0.5 mmol/kg of body weight. Even

10 more preferred is between 0.01 and 0.1 mmol/kg, and the most preferred dose of the active ingredient compound is between 0.02 and 0.05 mmol/kg.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be

15 required. Specific dosage regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion,

20 drug combination and the judgment of the treating physician.

It will be appreciated that the preferred pharmaceutical compositions are those comprising the preferred prodrugs of this invention.

25   In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not intended to be construed as limiting the scope of this invention in any manner. In each of the

30 examples, HSA is used as the protein to which bioactivated contrast agent binds.

EXAMPLES

Example Ia: Synthesis of Gadolinium Complex 1

First, carbamate **5** is reacted with the mono-
(diallylphosphate) ester of 4,4'-dihydroxybiphenyl in
the presence of diethylazodicarboxylate and triphenyl-
phosphine to form an ether (Scheme 2). Following
deprotection (TFA) and alkylation with bromo-t-
butylacetate, the phosphate is deprotected with
palladium tetrakis (triphenyl) phosphine. The t-butyl
esters are hydrolyzed with trifluoroacetic acid.
Reaction with $GdCl_3$ and sodium hydroxide produces the
gadolinium complex **1**.

Scheme 2: Synthesis of Gd Complex 1
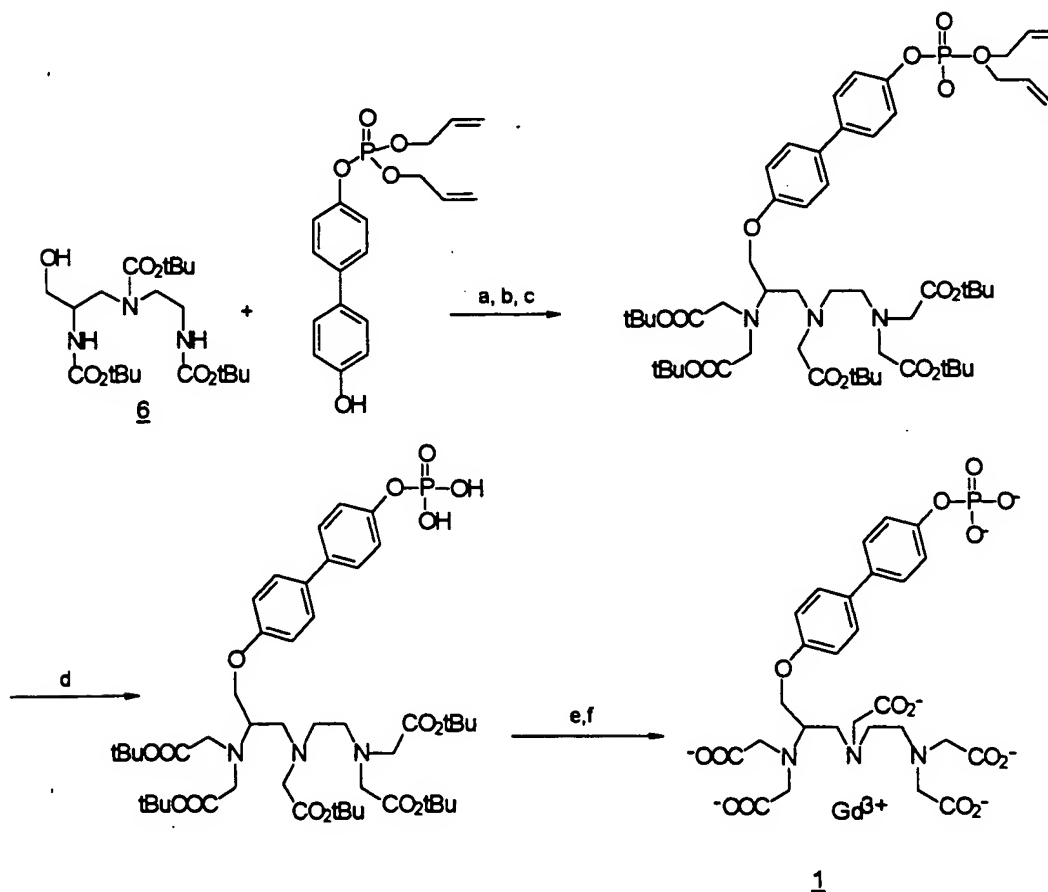
a) DEAD, PPh$_3$; b) TFA; c) BrCH$_2$CO$_2$tBu; d) Pd(Ph$_3$P)$_4$,n-BuNH$_2$,HCO$_2$H; e) TFA; f) GdCl$_3$, NaOH

Example Ib: More Preferred Synthesis of Gd Complex 1

First, boronic acid **13** was prepared in three steps from p-bromophenol (Scheme 2a). The phenol was protected as a tert-butyldimethylsilyl ether. Treatment of the arylbromide with butyllithium produced an aryllithium. Reaction of the aryllithium with triisopropyl borate followed by the hydrolysis of borate ester intermediate using mild acidic conditions produces boronic acid **13**.

Scheme 2a: Synthesis of boronic acid 13
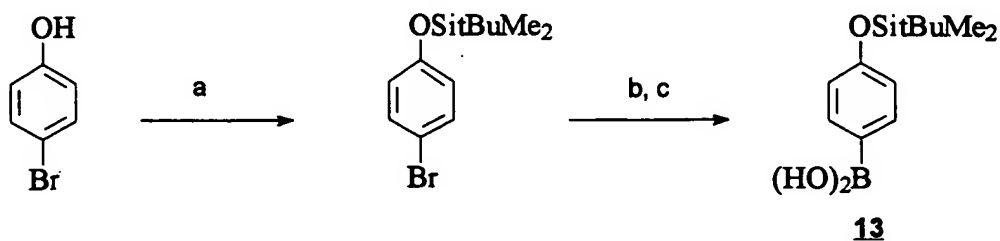
a) tBuMe$_2$SiCl, imidazole, THF; b) nBuLi, -78°C, THF: c) (i-PrO)$_3$B, THF then H$_3$O$^+$

Mitsunobu reaction between the intermediate **5** and bromophenol gave the ether **14**. Deprotection of the three BOC groups with trifluoroacetic acid followed by alkylation of the triamine with tert-butyl bromoacetate giving the pentaacetate **15**.
Tretrakis(triphenylphosphine) palladium-coupling (Suzuki coupling) of the arylbromide **15** with the boronic acid **13** resulted in the biphenyl derivative **16**. Hydrolysis of the silyl ether followed by phosphorylation and alkaline hydrolysis of the resulting phosphoryldichoride produced the monophosphate **17**. The t-butyl esters were deprotected

with trifluoroacetic acid. Reaction with GdCl₃ and
sodium hydroxide produced the gadolinium complex **1**
(Scheme 2b).

Scheme 2b: More Preferred Synthesis of Gd Complex 1
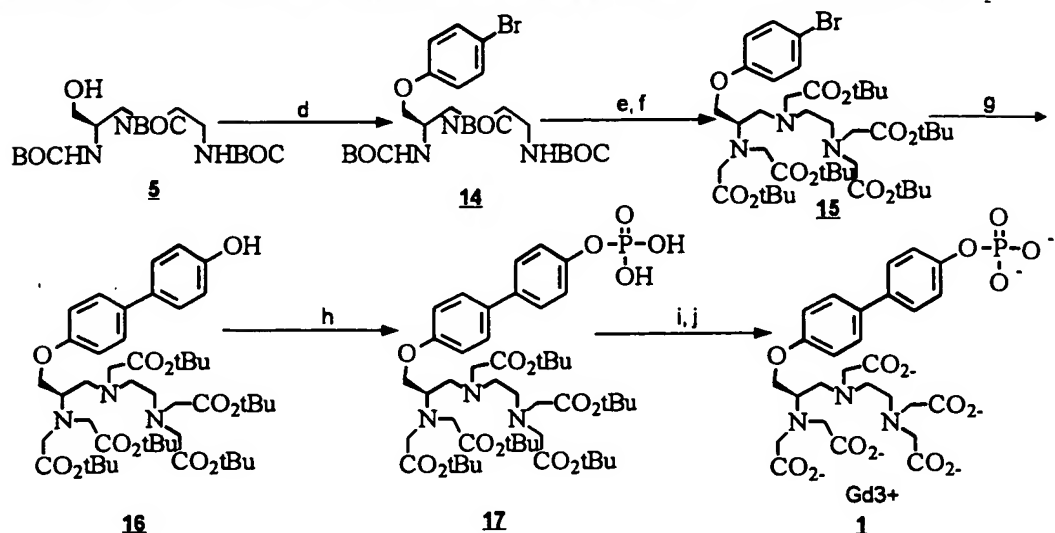
5   d) p-Bromophenol, DEAD, PPH₃ THF; e) TFA; f)
BrCH₂CO₂tBu, i-Pr₂NEt, DMF; g) **13**, (PPh₃)₄Pd, Na₂CO₃,
Toluene, MeOH, H₂O, Δ; h) POCl₃, Et₃N then H₂O/OH⁻; i)
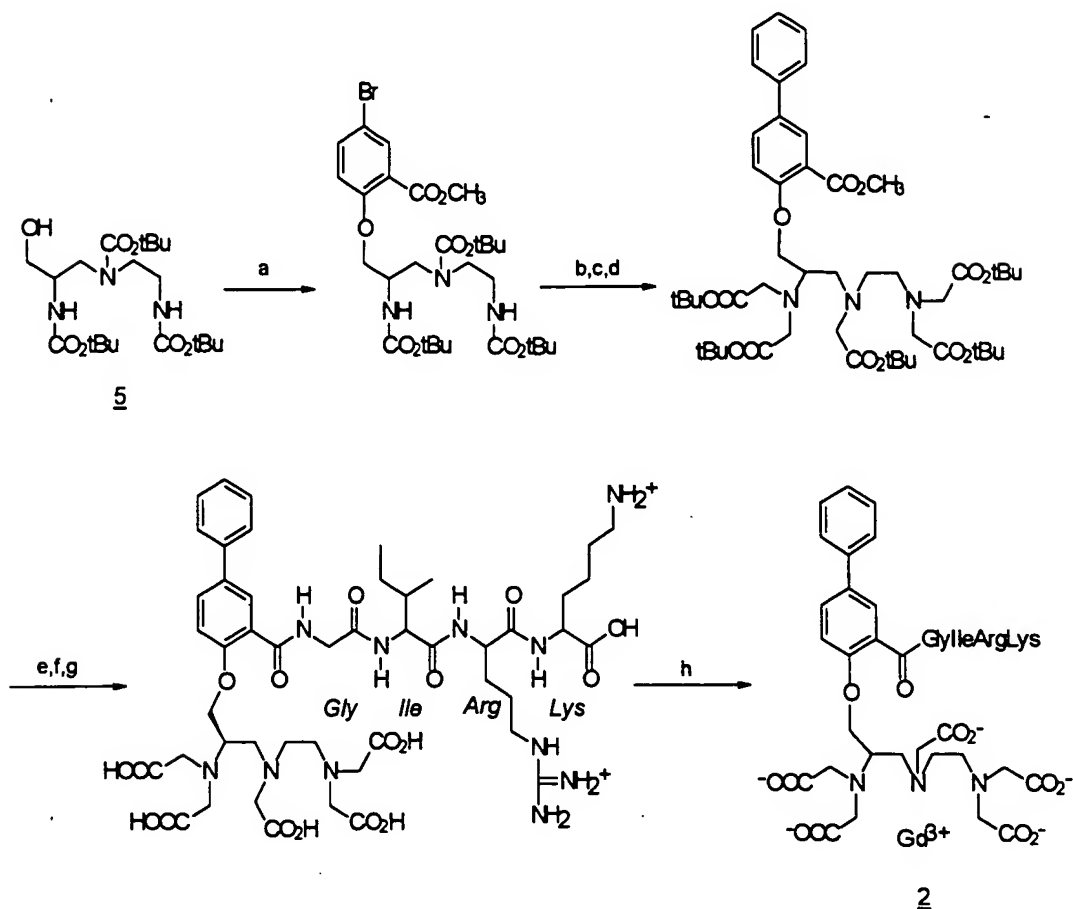TFA; j) GdCL₃, NaOH

Example IIa: Synthesis of Gadolinium Complex 2

10   Carbamate **5** is reacted with the methyl-5-
bromosalicylate in the presence of
diethylazodicarboxylate and triphenylphosphine to form
the bromoaryl ether. Tetrakis(triphenylphosphine)
palladium-catalyzed coupling of the bromoaryl ether

15   with phenylboronic acid affords the biphenyl ether.
Trifluoroacetic acid hydrolysis of the t-butyl
carbamates and subsequent alkylation with t-butyl
bromoacetate produces the biphenyl ether substituted
penta-t-butyl diethylenetriamine pentaacetate.

20   Hydrolysis of the methyl ester with 1 N NaOH in dioxane
gives the biphenylcarboxylate, which is coupled with

the peptide fragment H_2N-gly-ile-arg(Boc)$_2$-lys(Boc)-OtBu) using dicyclohexylcarbodiimide in dimethylformamide. Hydrolysis in 6N HCl/dioxane produces the biphenyl peptide substituted

5 diethylenetriamine pentaacetic acid. Reaction with GdCl$_3$ and base gives the gadolinium complex **2** which is purified by reverse-phase HPLC (Scheme 3a).

Scheme 3a: Synthesis of Gadolinium Complex 2

a) Methyl-5-bromosalicylate, DEAD, Pph$_3$; b) ArB(OH)$_2$, Na$_2$CO$_3$, (Ph$_3$P)$_4$Pd; c) TFA; d) BrCH$_2$CO$_2$tBu, iPr$_2$NEt; e) NaOH, H$_2$O/dioxane; f) H$_2$N-gly-ile-arg(boc)$_2$-lys(boc)-OtBu, DCC; g) HCl/dioxane; h) GdCl$_3$, base.

Example IIb: More Preferred Synthesis of Gd Complex 2

Carbamate **5** was reacted with methyl-5-bromosalicylate in the presence of diethylazodicarboxylate and triphenylphosphine forming a bromoaryl ether. Hydrolysis of the methyl ester followed by treatment with benzylchloroformate and

triethylamine in the presence of a catalytical amount
of dimethylaminopyridine resulted in the benzyl ester
18. Deprotection of the three BOC groups with
trifluoroacetic acid and subsequent alkylation with
5   tert-butyl bromoacetate produced the bromoaryl ether
substituted penta-tert-butyl diethylenetriamine
pentaacetate **19**. Suzuki coupling of bromoaryl ether
with phenylboronic acid gave the biphenyl ether.
Hydrogenolysis of benzylester in the presence of
10 palladium catalyst gave the biphenylcarboxylate **20**.
Coupling of **20** with benzyl glycinate followed by the
hydrogenolysis of the benzyl ester gave the amide **21**.
Coupling of **21** with the protected tripeptide, H_2N-Ile-
Arg(BOC)$_2$-Lys(BOC)-OtBu, using dicyclohexylcarbodiimide
15 in DMF and subsequent deprotection of the t-butyl
esters and the BOC groups with TFA resulted in the
tetrapeptide **22**. Reaction with GdCl$_3$ in the presence of
sodium hydroxide gave complex **2** which is purified by
reverse-phase HPLC (Scheme 3b).

Scheme 3b: More Preferred Synthesis of Gd Complex 2
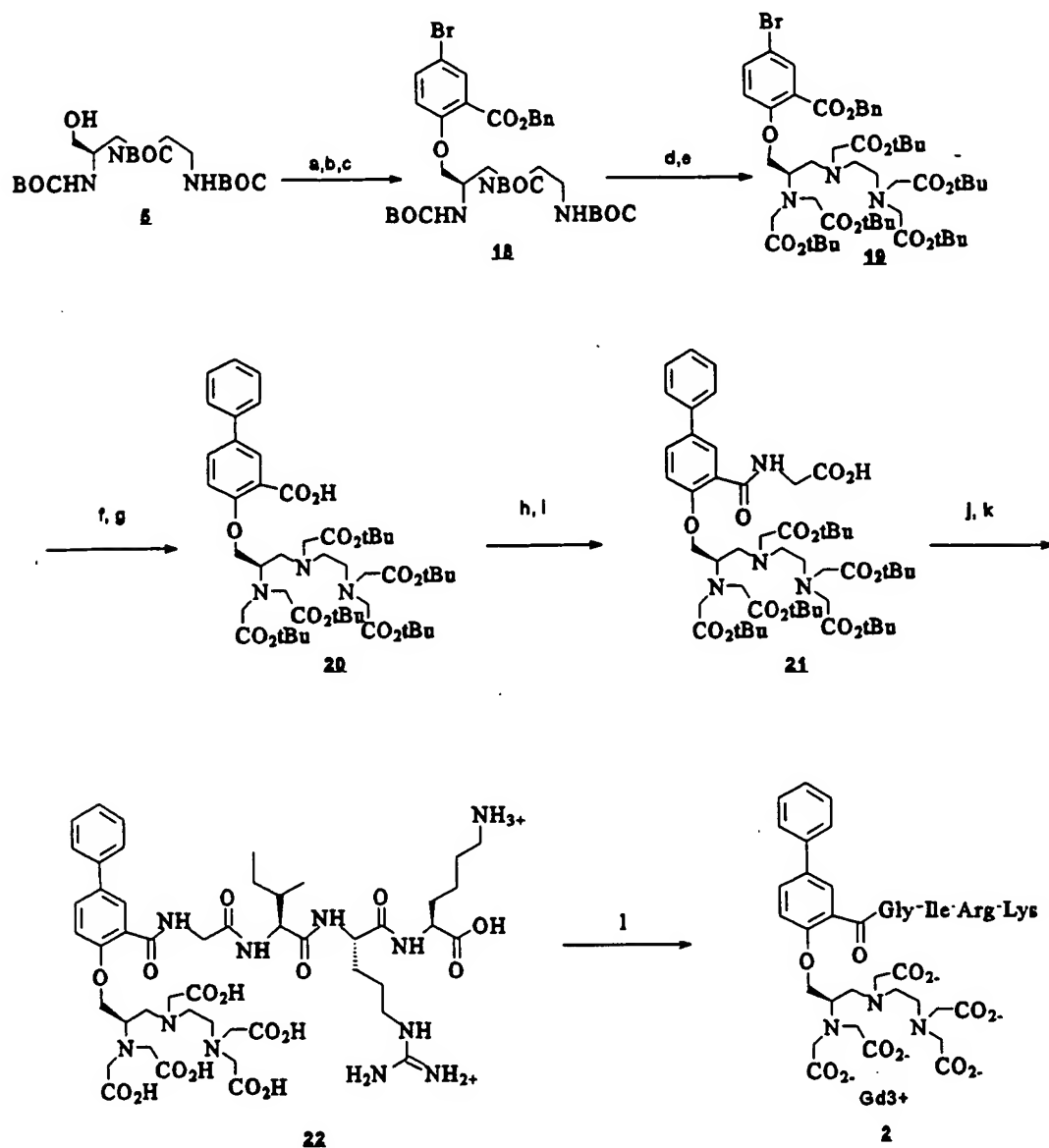
a) Methyl-5-bromosalicylate, DEAD, PPh_3, THF; b) 2N KOH
(aq), MeOH; c) $ClCO_2Bn$, Et_3N, DMAP, CH_2Cl_2; d) TFA;
e) $BrCH_2CO_2tBu$, i-Pr_2NEt, DMF; f) Ph-B(OH)$_2$, $(Pph_3)_4Pd$,
Na_2CO_3, Toluene, MeOH, H_2O,Δ; g) H_2, Pd, C, MeOH; h)

5    $H_2NCH_2CO_2Bn$, DCC, CH_2Cl_2; 1) H_2, Pd, C, MeOH; j) H_2N-113-
Arg(BOC)$_2$-Lys(BOC)-OtBu, DCC, HOBt, DMF; k) TFA;
i) $GdCl_3$, NaOH.

Example IIIa: Activation of Prodrug Compound 1

Prodrug **1** is activated by alkaline
10   phosphate as shown below. Activated contrast agent **8**,
produced by hydrolysis of the MS (phosphorous-oxygen
bond), binds at a concentration of 0.1 mM to HSA with
greater affinity than prodrug **1**. The increased
relativity results from a shortening of T_1, which is
15   detected as an increase in signal intensity in an MRI
image.

Activation of Prodrug Compound 1
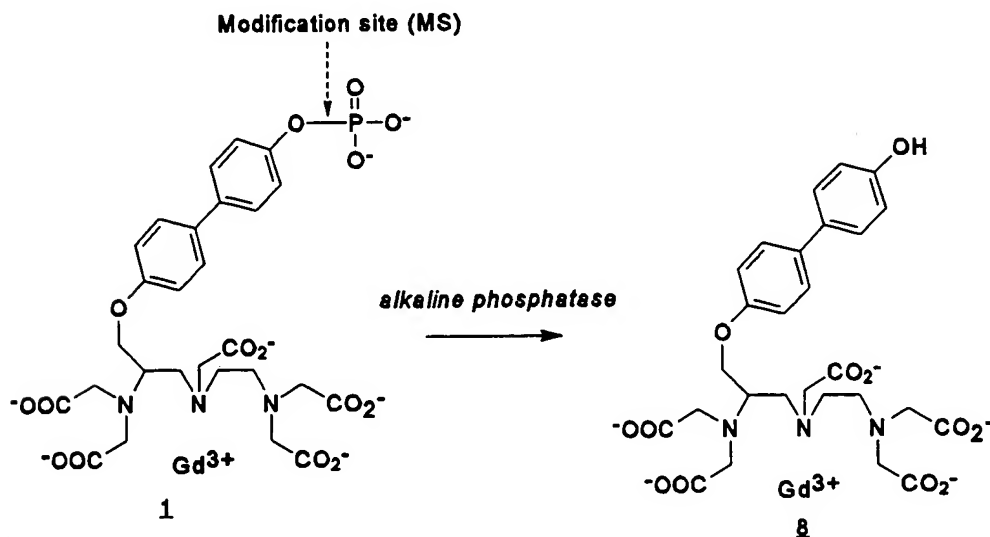
**Example IIIb: More Preferred Activation
of Prodrug Compound 1**

Compound **1** (see Examples Ib and IIIa) was
synthesized as described in Example Ib. Prodrug **1** was
5   activated by alkaline phosphatase (see Example IIIa⁻
Activation of Prodrug **1**) which hydrolyses the MS
(phosphorus-oxygen bond), forming activated contrast
agent **8**. Compound **8** bound at a concentration of 0.1 mM
to HSA with greater affinity than prodrug **1** and with a
10   higher relaxivity. The higher relaxivity resulted from
a shortening of T_1, which was detected as an increase
in signal intensity in an MRI image (See Table V
below).

TABLE V

15   Relaxivity and Percent Binding to HSA

Compound	Relaxivity in 4.5% HSA	Percent Binding in 4.5% HSA
1	15.9 ± 0.2	47 ± 1
8	26.4 ± 0.4	63 ± 2

In Table V, the longitudinal relaxivities
20   (R_1) were obtained at 20 MHz and 37°C by determining the
concentration dependent relaxation rate ($1/T_1$) of water
protons in phosphate buffered saline (PBS, 150 mM NaCl,
10 mM phosphate, pH=7.4) or in PBS solutions containing
4.5% human serum albumin (HSA). The percent bound to
25   HSA was determined by ultrafiltration of a 0.1 mM
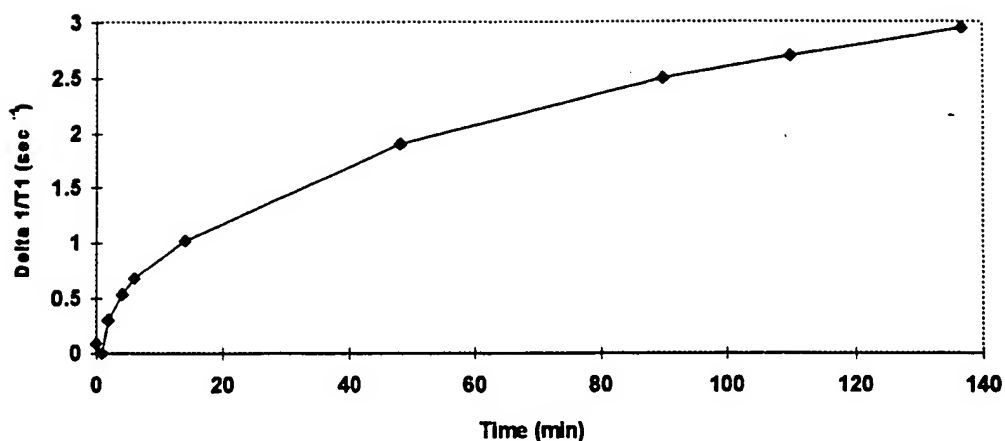chelate, 4.5% HSA solution.

A solution of prodrug **1** (0.3mM) was prepared
in PBS buffer (pH 7) containing 4.5% HSA. No change in
the 20 MHz proton relaxation rate $1/T_1$ was observed
30   with time. Three units of alkaline phosphatase (1 unit

converts 1 nmol of p-nitrophenylphosphate to p-nitrophenol per minute in phosphate buffered saline at 0.1 mM substrate) were added to the 4.5% HSA solution of compound **1** and the $1/T_1$ was monitored over time.

5 The $1/T_1$ for the solution was observed to change as **1** was enzymatically converted to **8** (Table VI). Upon completion of enzymatic activation of **1** to **8**, the change in $1/T_1$ from time zero was 2.86 sec^{-1}, which corresponds to an approximate expected increase in

10 signal intensity of 24%.

TABLE VI
Bioactivation of Prodrug 1 at 20 MHz

| Time (min)	$1/T1$ (sec^{-1})
0	4.440
1	4.348
2	4.651
4	4.878
6	5.025
14	5.376
48	6.250
90	6.849
110	7.042
137	7.299

15

20

Solutions of 4.5% HSA containing compounds **1** and **8** (0.1-0.2 mM) were prepared. After 15 minutes, an initial T1-weighted MRI scan (FISP-3D, TR=60, TE=5, alpha=60) at 1.0 Tesla of the 4.5% HSA solutions was

5   obtained. The MRI scans of the solutions containing **8** were brighter than the solutions containing **1** at equivalent concentrations. Three units of alkaline phosphatase were added to half of the HSA solutions containing **1** and additional T1-weighted MRI scans were

10   obtained. After 130 minutes, the solutions that contained **1** and alkaline phosphatase obtained 96% of the signal intensity that for the solutions that contain **8** at equivalent concentrations. The solutions that contained **1** without alkaline phosphatase remained

15   as constant dark images during the MRI scans. A 20% increase in signal intensity was observed after addition of alkaline phosphatase to solutions containing **1**.

Example IV: Activation of Gadolinium Complex 2

Prodrug **2** containing a hydrophilic
isoleucine-arginine-lysine side chain MM is activated
by collegenase (MMP-1). The MS is a carbon-nitrogen
5   peptide bond which is selectively hydrolyzed by MMP-1
(gly-ile). Release of the ile-arg-lys MM generates
compound **9,** which is characterized by higher binding
affinity for HSA than the prodrug **2.** The altered
signal in the MRI permits the bioactivity to be imaged.

10   Activation of Gadolinium Complex 2

**2**   **9**
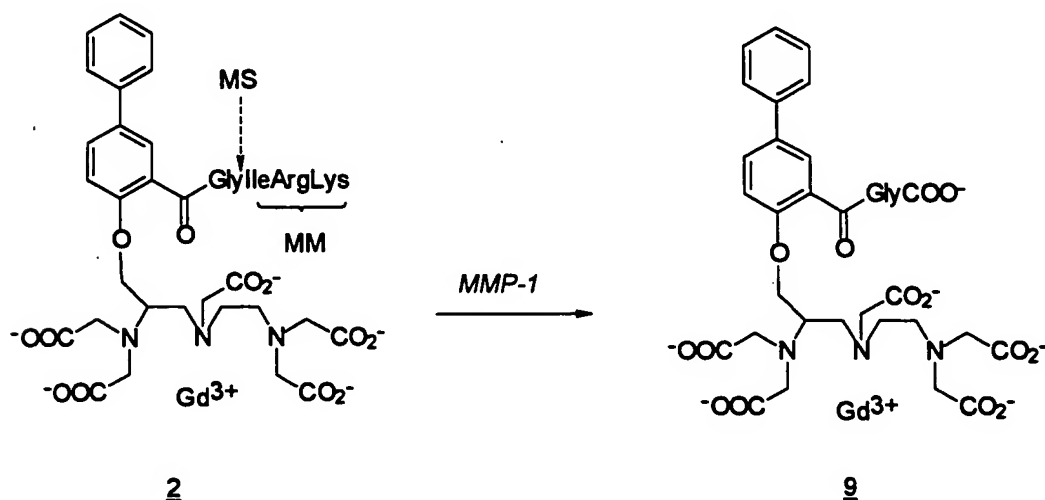
Example V: Activation of Gadolinium Complex 10

This example also shows how a secondary
chemical reaction is coupled to a primary bioactivity-
related event. Prodrug **10** containing an MM composed of
15   the tripeptide tmLys-tmLys-Arg (where tmLys is Nε, Nε,
Nε-trimethylysine) is activated by serine protease.
The MS is the Arg-Glu carbon-nitrogen peptide bond
which is cleaved by serine proteinase enzymatic
bioactivity to release the masking moiety. The
20   enzymatic hydrolysis to give intermediate compound **11**
is followed by a secondary chemical reaction

(intramolecular cyclization) with an aliphatic or
activated ester (e.g., R=p-nitrophenyl). This converts
the positively charged PBM moiety in **11** to the more
lipohilic, more highly HSA-bound, neutral lactam

5   derivative **12**.

Activation of Prodrug Compound 10

10   Intermediate **11**

cyclization

12
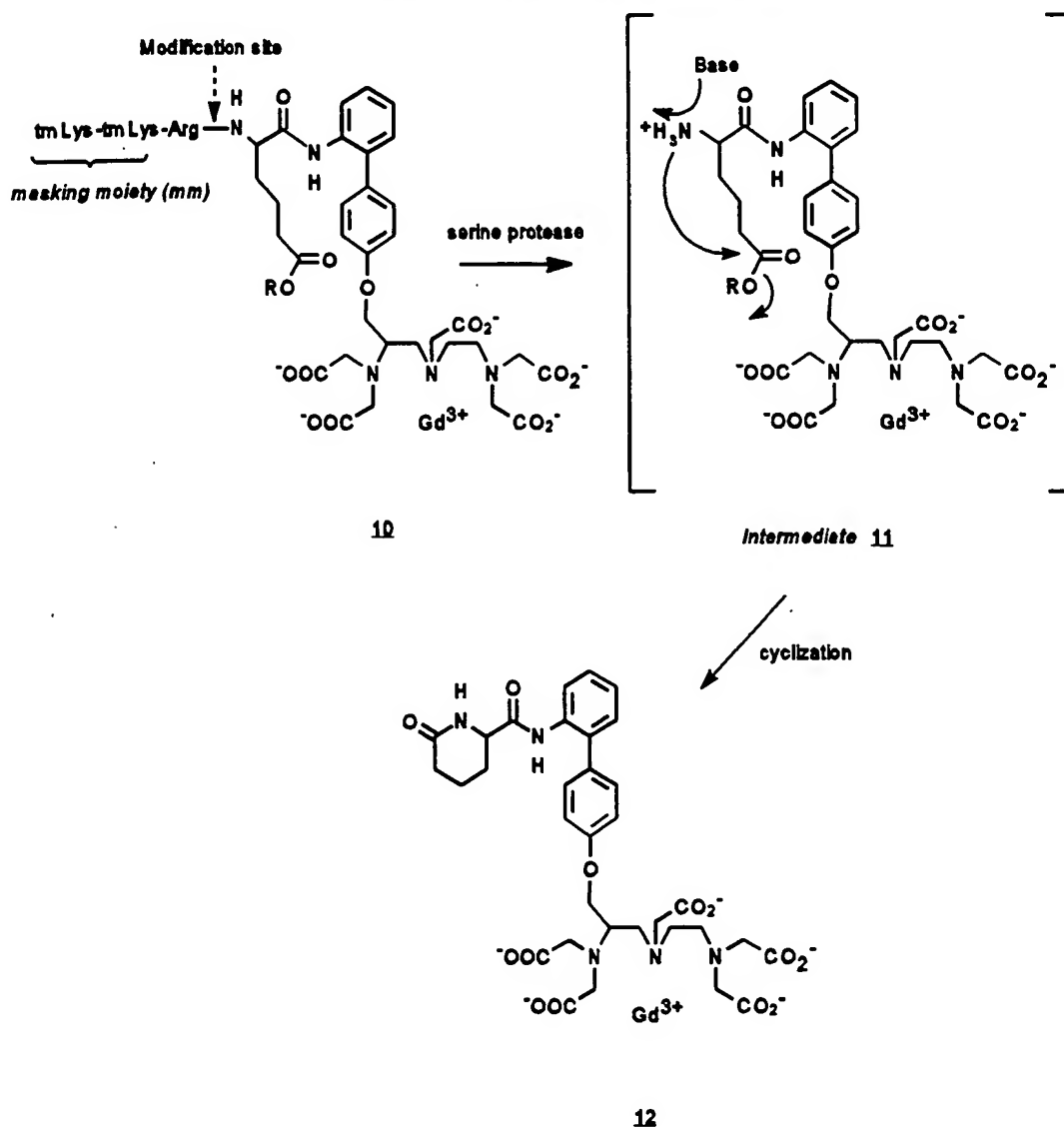
Example VI: Administration of Prodrug Contrast Agents

Prodrug compound **2**, an MMP substrate, is
synthesized by chemical and peptide techniques known in

the art and described above. A pH 7.0 formulation in water is prepared and sterilized.

The formulation is injected intravenously into a patient suspected of having one or more tumors.

5 The dosage used is 0.025 mmol/kg. After 15 minutes - post-injection, T_1-weighted MRI scans of the region of the body suspected to contain tumors is obtained. Masses that appear bright on the images are more likely to be malignant tumors than benign.

10 This same formulation is injected intravenously into a patient suspected of having rheumatoid arthritis in one or more joints. The dosage used is 0.025 mmol/kg. After 15 minutes post-injection, T_1-weighted MRI scans of the joints of the

15 body suspected to contain arthritis is obtained. Joints that appear bright on the images are more likely to contain active inflammation.

This same formulation is injected intravenously into a patient with atherosclerosis in

20 one or more arteries. The dosage used is 0.025 mmol/kg. After 15 minutes post-injection, T_1-weighted MRI scans (cross-sectional scans or MR angiography) of the arteries is obtained. Atherosclerotic plaque that enhances brightly is more likely to be unstable plaque,

25 likely to rupture and cause ischemia to the organ the artery feeds.